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ARCHIVES OF PATHOLOGY

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STRUCTURE AND HISTOGENESIS OF TUMORS OF THE AORTIC BODIES IN DOGS

WITH A CONSIDERATION OF THE MORPHOLOGY OF THE AORTIC AND CAROTID BODIES

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FLUSHING, L. I., N. Y.

Tumors located in the region of the base of the heart, i. e., in the cardiac mediastinum, have been described in dogs and other species, as horses, oxen, sheep and fowl. They are unrelated to the neoplasms of the heart which have been reviewed in the domesticated animals by Magnusson [1] and Mossdorf.[2] The tumors at the base of the heart do not constitute a pathologic entity any more than mediastinal tumors in man do. The term "base of the heart" simply indicates the anatomic location of the neoplasms, although this designation is not commonly employed in human pathology. Barth [3] in 1920 reviewed the literature concerning such tumors in dogs and described a variety of neoplasms. Individual reports of growths thus located in dogs are given by Wetzel,[4] who mentioned a mixed cell sarcoma, Joest,[5] who noted a thymoma, and Viergutz,[6] who observed a cancerous teratomatous epithelial neoplasm. Nieberle and Cohrs [7] stated that the accessory thyroid glandules which frequently occur in the subpericardial periaortic connective tissue of dogs may possibly give rise to the so-called heart base tumors. Jackson [8] described 3 neoplasms of identical morphologic characteristics with this location in dogs. He was impressed with the histologic resemblance of these tumors to the canine transmissible venereal lymphosarcoma and considered them as neuroblastoma or as sympathogonioma that arose from neuroblastic elements (sympathogonia or early sympathoblasts) present between the aorta and the pulmonary artery. With this theory there consequently developed the corollary that the transmissible sarcoma was also neuroblastoma (sympathogonioma).

In a series of 500 necropsies 2 tumors were encountered in the region of the base of the heart that had the same gross and microscopic anatomy as those described by Jackson. Although in their general histologic features these growths somewhat resembled the venereal tumors, certain cytologic and structural differences were noted. However, these growths had none of the morphologic characteristics of

1. Magnusson, H.: Ztschr. f. Krebsforsch. **15**:212, 1916.
2. Mossdorf: Ztschr. f. Veterinärk. **42**:409, 1930.
3. Barth, A.: Ein Beitrag zur Kenntnis der Herzbasisgeschwülste beim Hunde, Inaug. Dissert., Dresden, O. Franke, 1920.
4. Wetzel, R.: Ueber ein Sarkon an der Herzbasis beim Hunde, Inaug: Dissert., Hanover, E. Jurgens, 1922.
5. Joest, E.: Spezielle pathologische Anatomie der Haustiere, Berlin, R. Schoetz, 1924, vol. 3, p. 1.
6. Viergutz, H. E.: J. Am. Vet. M. A. **101**:490, 1942.
7. Nieberle, K., and Cohrs, P.: Lehrbuch der speziellen pathologischen Anatomie der Haustiere, Jena, Gustav Fischer, 1931, p. 12.
8. Jackson, C.: Onderstepoort, J. Vet. Sc. & Animal Indust. **6**:11, 1936.

neuroblastoma, so that an origin from the sympathetic nervous system is questionable. Microscopic study of the normal aortic bodies located in the region of the base of the heart demonstrated that the tumors originated from these structures. As there has been general agreement that the epithelioid cells of these bodies are identical with the cells of the carotid body, the tumors under discussion were compared with tumors of the carotid body.

A review of the literature on the cardiac and mediastinal tumors of man indicates that no mention has been made of neoplasms of the aortic bodies or of the fact that these structures may undergo neoplastic proliferation. As the bodies in man and dog are similar in location and appearance, it is conceivable that tumors which occur in this region in man are identical with those in dogs, particularly when they appear in the cardiac mediastinum and present histologic features that are atypical of the usual neoplasms found in this region.

MATERIAL AND METHODS

The tumors were obtained from dogs brought to my animal hospital for treatment. The tissues were fixed in Zenker formaldehyde solution (Zenker's stock solution with addition of solution of formaldehyde U. S. P.) and in 10 per cent solution of neutral formaldehyde U. S. P. For the demonstration of the aortic bodies, 3 puppies 12 hours old were killed with soluble pentobarbital, given intraperitoneally, and in 2 instances the heart with the attached vessels was fixed in each of the aforenamed solutions. In the third, fixation was accomplished in a 25 per cent solution of chloral hydrate in 50 per cent alcohol for subsequent staining of the nerve fibers by the Cajal method as modified by Nonidez.[9] I received from Dr. N. C. Foot unstained slides of a tumor of a human carotid body, from Dr. E. L. Stubbs a paraffin block and unembedded tissue of a transmissible sarcoma and from Dr. J. Furth slides of a venereal tumor, stained with hematoxylin and eosin.

My sections were stained with hematoxylin and eosin, Van Gieson's stain, Masson's[10] trichrome stain, Dominici's stain,[11] Mallory's phosphotungstic acid–hematoxylin, Wilder's[12] reticulum stain and Verhoeff's elastica stain and by Bodian's[13] strong protein silver method. Formaldehyde-preserved tumor and transmissible sarcoma tissue was stained for nerve fibers following the suggestions of Nonidez[14] as to the application of his modified Cajal method to such material. Sections of the tumors, aortic bodies and adrenal glands of the same animals previously fixed in Zenker formaldehyde solution were mordanted for four days in 5 per cent potassium dichromate solution to demonstrate the possible presence of the chromaffin reaction.

GENERAL OBSERVATIONS

The tumors occurred in male Boston terriers 14 years (case 1) and 9 years old (case 2). There were no outstanding clinical symptoms with the exception of cardiac insufficiency in both dogs and generalized edema, ascites and hydrothorax in the 9 year old dog. Laboratory examination showed the blood and the urine to be essentially normal. Histologic study of all tissues and organs revealed no pertinent findings except advanced chronic passive congestion of the liver in the 9 year old dog. Metastatic tumor formation was absent in both animals.

MACROSCOPIC ANATOMY

CASE 1.—Occupying the region at the base of the heart was a somewhat dumbbell-shaped growth, 8 cm. long, 4 cm. thick at its widest and 1.8 cm. thick at its narrowest point (fig. 1 *A* and *B*). It produced a marked depression of the atriums, which were compressed by the tumor above and the ventricles below. Both the aorta and the pulmonary artery were

9. Nonidez, J. F.: Am. J. Anat. **65**:361, 1939.
10. Masson, P.: J. Tech. Methods **12**:75, 1929.
11. McClung, C. E.: Handbook of Microscopical Technique, New York, Paul B. Hoeber, Inc., 1937, p. 340.
12. Wilder, H. C.: Am. J. Path. **11**:817, 1935.
13. Bodian, D.: Anat. Rec. **69**:153, 1937.
14. Nonidez, J. F.: Personal communication to the author.

partly enveloped by the growth, which was grooved to accommodate both vessels. The tumor was a mottled reddish brown and had a firm consistency on palpation. The surface was slightly nodulated and was covered with a thin capsule that was thicker in areas. On section, varying-sized trabeculae were irregularly distributed throughout the growth.

CASE 2.—In the region at the base of the heart was a roughly triangular mass that greatly compressed both atriums. Its broad base, which measured 6.2 cm. in diameter, was in intimate contact with the heart itself. It extended dorsally for 8.3 cm. and was grooved to invest the great blood vessels completely. The superior vena cava, the trachea, the bronchi and the esophagus were partially surrounded by the new growth. The surface consisted of smaller and larger nodules and was covered with an irregularly thickened capsule. On section, trabeculae of different thicknesses penetrated the firm, brownish red tumor tissue.

MICROSCOPIC ANATOMY

Numerous sections from different portions of each tumor showed similar histologic characteristics (fig. 2*A* and *B*). In suitable areas a fibrous connective tissue capsule that was

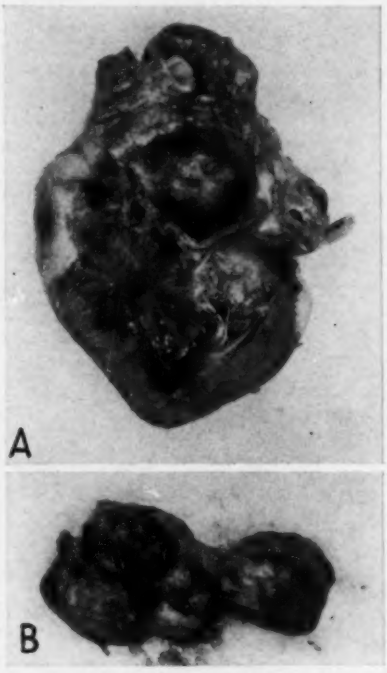

Fig. 1.—*A*, gross appearance of the tumor at the base of the heart in case 1 after removal of part of the ventricular wall; × ½. *B*, the tumor mass dissected out; × ½.

usually covered with epicardium invested the tumor tissue. In regions the capsule was thick, but usually it was thin and consisted of several concentric layers of adult connective tissue fibers. Appearing to originate from the capsule and extensively penetrating the growth were many irregularly distributed collagenous trabeculae. They varied in width from those that were thin and delicate to others that were thick and dense. The latter were occasionally hyalinized and in several areas showed cholesterol clefts and hemosiderin pigmentation. In some regions the capsule and the trabeculae were infiltrated with neoplastic cells. The septums were usually highly vascularized, the blood vessels being of all calibers, and further branched into thinner strands of more delicate collagenous fibers. The distribution of the stromal tissue was decidedly irregular and roughly served to divide the tumor parenchyma into smaller and larger pseudolobular formations. Reticulum stains demonstrated regional variations in the number of argyrophilic fibers that tended to invest groups of cells with no intercellular distribution (fig. 3*A*). Special stains revealed complete absence of elastic connective tissue and nerve fibers.

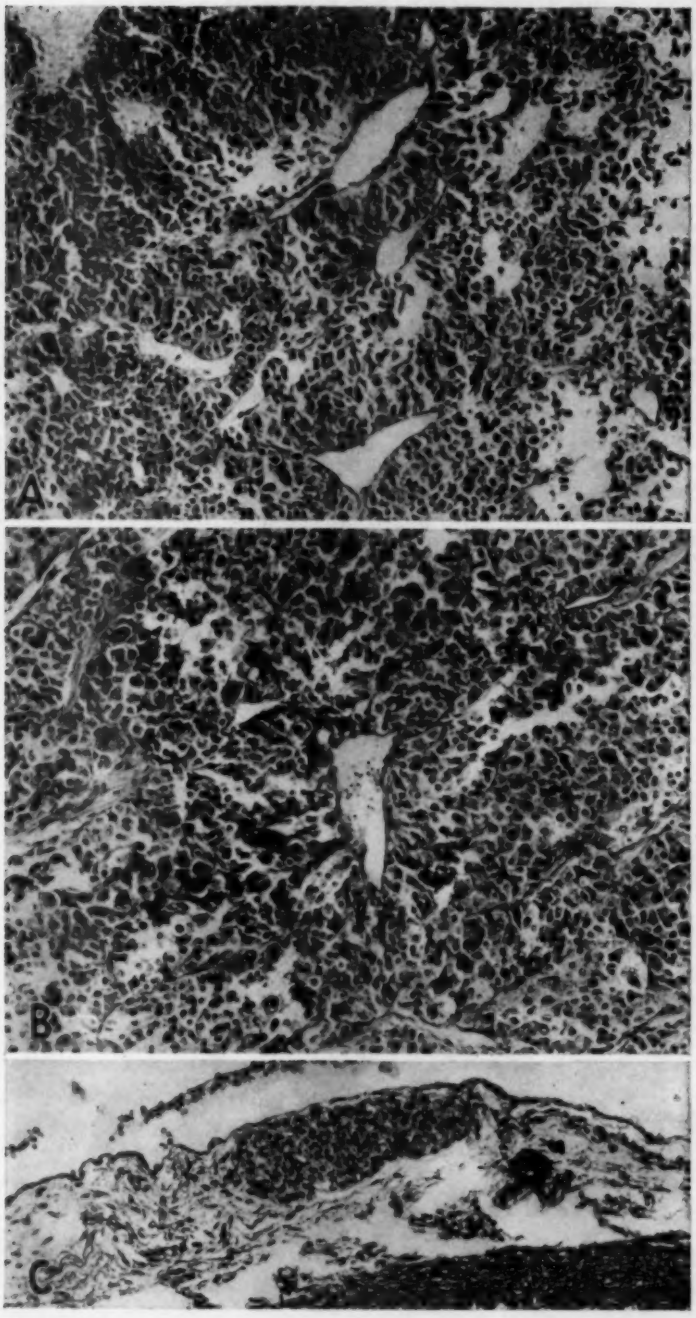

Fig. 2.—*A*, photomicrograph of the tumor in case 1 showing the peritheliomatous formations, the vascularity and the cellular arrangements. In the upper left corner is an area of necrosis. Hematoxylin and eosin; × 120.

B, photomicrograph of the tumor in case 2 showing features similar to those of the tumor in case 1 (*A*), although there are greater variations in cellular size. Hematoxylin and eosin; × 120.

C, photomicrograph of an aortic body located in the adventitia of the pulmonary artery of a puppy; × 120.

The tumor cells were round, oval or polyhedral (fig. 4*A*). The ample cytoplasm was acidophilic and finely granular. The cellular borders were indistinct, but cytoplasmic streaming and polymorphism were not exhibited. The nucleus had a distinct membrane and a rich chromatin network that was usually aggregated into fine and coarse granules. It was round or oval, occasionally indented or elongated, and contained a single central or eccentric eosinophilic nucleolus. The nucleus usually occupied the central portion of the cell but was often peripherally located. While the majority of cells exhibited only slight variations in size and presented a uniform appearance, large mononucleated cells were irregularly

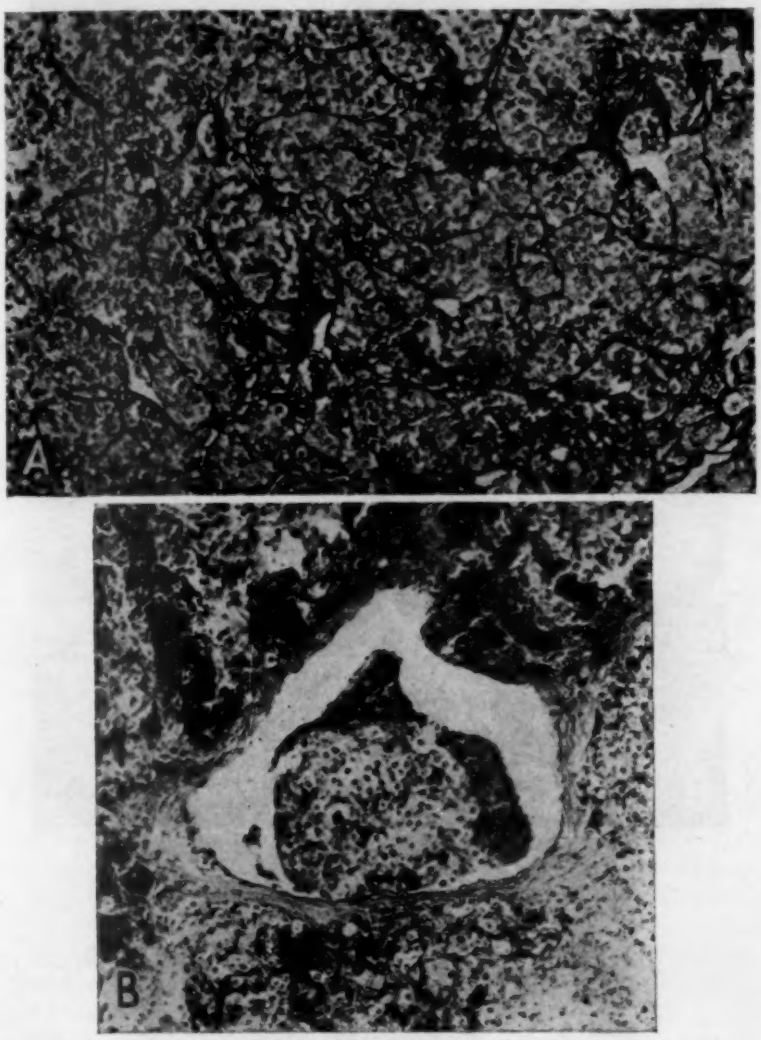

Fig. 3.—*A*, photomicrograph of the tumor in case 2 illustrating the argyrophilic network. In other regions reticulum fibers were less profuse. Wilder's reticulum stain; × 120. *B*, photomicrograph of the tumor in case 2 depicting intravascular tumor cells. The darker staining masses in the upper portion of the figure represent hyperchromatic nuclei in a deeply eosinophilic syncytial cytoplasmic matrix. Hematoxylin and eosin; × 120.

distributed in areas. These giant cells were often oval, spherical or polyhedral, but many times they assumed bizarre shapes (fig. 4*B*). Their cytoplasm was frequently deeply eosinophilic and the large hyperchromatic nucleus contained from one to three acidophilic nucleoli. The giant cells occurred in both tumors but were more numerous in that in case 2, which

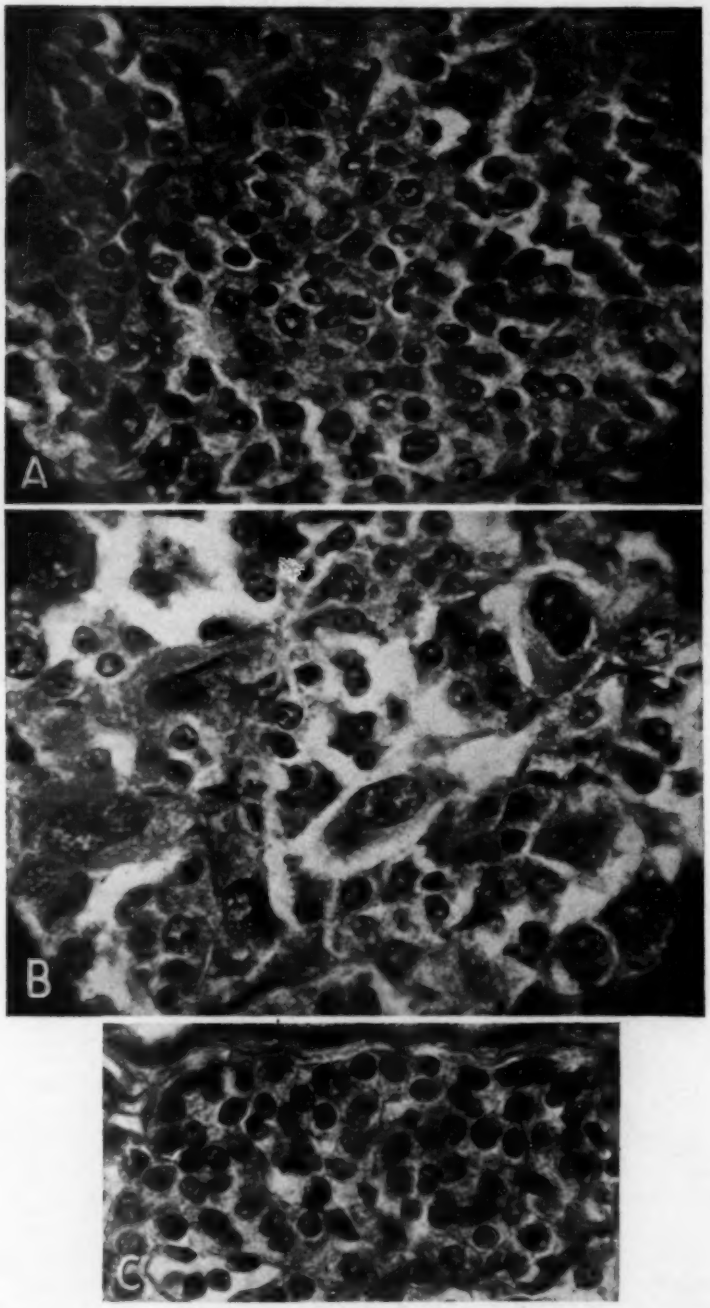

Fig. 4.—*A*, photomicrograph of the tumor in case 1 showing cellular details. Hematoxylin and eosin; × 600.

B, photomicrograph of the tumor in case 2 taken from an anaplastic field showing giant cells and cellular pleomorphism. Hematoxylin and eosin; × 600.

C, photomicrograph of the aortic body depicted in figure 2*C*. Observe the morphologic similarities to the tumor cells in *A* and the smaller cells in *B* of this figure. Hematoxylin and eosin; × 600.

appeared more anaplastic. A few cells were binucleated, and mitotic figures were present only occasionally. The tumor cells gave a negative chromaffin reaction in contrast to the positive chromaffininity of the cells of the adrenal medulla used as a control.

The arrangement and distribution of the neoplastic cells varied in different areas of even the same tumor. Most commonly, the cells were closely packed and occurred in larger and smaller sheetlike masses and groups separated by the septums. They often assumed a somewhat alveolar formation without any central lumen, and occasionally lobular arrangements occurred. Ball-like masses, pseudorosettes and true rosettes were completely absent. In both cases the tumor cells frequently presented a distinct peritheliomatous appearance by their radial arrangement about blood vessels of all calibers. This feature was most marked in case 1 (fig. 2*A*). A rich capillary network permeated the neoplasm and in many areas represented the chief stromal constituent, connective tissue being minimal. The blood vessels were frequently congested, and smaller and larger hemorrhages occurred in some regions. Occasional small areas of necrosis, unaccompanied by any cellular reaction, were present.

In irregularly distributed regions there occurred varying-sized groups of hyperchromatic nuclei, with many of giant size, in a deeply eosinophilic syncytial cytoplasmic matrix. Cell boundaries were indistinguishable, and stroma and blood vessels were absent, though occasional small hemorrhages occurred. In some areas the syncytial masses were separated into irregular columns by an intervening capillary network or fibrous stroma. Among the tumor cells were occasional oblong, elongated or triangular cells with deep eosinophilic homogeneous cytoplasm and a hyperchromatic nucleus. Many times these appeared to be more prominent around capillaries and larger blood vessels.

In both tumors, groups of neoplastic cells were present in the blood vessels and lymphatics (fig. 3*B*). Often the intravascular tumor cells appeared as a syncytial mass with hyperchromatic nuclei and occasionally were adherent to the intima.

There was a complete absence of lymphocytes, mast cells, eosinophils, plasma cells and polymorphonuclear leukocytes in the stroma and the parenchyma.

Sections taken through the junction of the tumor with the regional lymph nodes, trachea, bronchi, esophagus, aorta, pulmonary artery and other blood vessels indicated no invasion of these structures. Sections from the point of contact of the neoplasm with the atriums showed superficial infiltration of the cardiac muscle by tumor cells.

COMMENT

These 2 similar primary neoplasms with location in the region of the base of the heart and with unusual histologic characteristics immediately presented the problem of diagnostic classification. It is unnecessary to enumerate the many probable sites that these growths could be derived from, as they showed no resemblance to the various neoplasms that are known to occur in the intrathoracic cavity. They were not metastatic tumors, as no primary foci were found in a careful histologic survey of all tissues and organs. In addition, the microscopic features were not significant for any tumor previously described. In the search for a possible source of the tumors in the region at the base of the heart a consideration of the normal structures in this region indicated that the aortic bodies showed a remarkable similarity to the neoplasms. It is therefore assumed that the tumors originated from these structures, not only because of the morphologic likeness but, in addition, because of the improbability of other sources. The question of histogenesis will be further discussed following the description of the aortic bodies.

In the light of recent investigations a reexamination of the histologic status of the aortic bodies (and the carotid body) is necessary to establish the identity of these cell groups so that tumors of these structures can be properly classified. In the base of the heart near the coronary arteries, in tissue dorsal, ventral and lateral to the aortic arch, in tissue between the aorta and the pulmonary artery and in tissue near the root of the right subclavian artery are inconspicuous groups of epithelioid cells that have been termed paraganglion caroticum inferius by

Rabl,[15] paraganglion aorticum supracardiale by Penitschka,[16] paraganglion supracardiale superius and inferius by Palme,[17] aortic glomi by Muratori,[18] supracardial paraganglia, aortic paraganglion and aortic glomi by Nonidez [19] and aortic arch bodies by Boyd.[20] Hammond's [21] suggestion that Boyd's [20] term should be used to identify all such groups of cells appears justified since they are essentially alike in structure and innervation. The carotid body can also be included under this designation as it is identical with these epithelioid cell groups. The distribution of the aortic bodies is similar in man and the dog and differs from that in the rabbit and the cat.[19c]

Nonidez [19] made a morphologic study of the aortic bodies in various animals and found only minor variations in the different species. In the dog,[22] their structure is very uniform, and they are enclosed within a thin connective tissue capsule (fig. 2*C*). The epithelioid cells are of a single type with only slight variations in size (fig. 4*C*). The cytoplasm is slightly granular, and the round or oval nucleus has a fine chromatin network with one or more nucleoli. The cellular borders are indistinct. The cells are usually closely packed and may form irregular strands separated from each other by capillaries. Arterioles are numerous, and there is a rich capillary network. Connective tissue stains reveal a sparse reticulum, principally confined to the pericapillary region. The capillary walls are thin with few nuclei, and many epithelioid cells seem to be in direct contact with the endothelium, a condition also observed in the carotid body by de Castro.[23] The nerve supply is very abundant and consists of small nerve fibers which break into numerous branches, some of which form pericellular baskets. The finer nerve radicals end as minute rings and small club-shaped enlargements closely applied to the cellular cytoplasm.

The histologic structure of the carotid body and of the aortic bodies has been variously interpreted by different investigators. It has been generally agreed that the carotid body and its tumors are morphologically characteristic of the paraganglionic system.[24] This concept is largely due to Kohn's [25] establishment of the paraganglions as a tissue system, of which the carotid body has been considered a member. Trinci [26] and Busacchi [27] included the aortic bodies among the paraganglions. The criteria by which the paraganglions are identified consist of a common embryonic origin from the sympathetic system, the presence of a true chromaffin reaction, the secretion of epinephrine or some similar pressor substance and, as Hollinshead [28] recently showed, the reception of efferent nerve fibers. Nonidez [19a] could not demonstrate chromaffin cells in the aortic body of the rabbit or that of the guinea pig, although scattered yellowish cells occur in the cat. The cells in the dog do not give a positive chromaffin reaction. Smith [29]

15. Rabl, H.: Arch. f. mikr. Anat. **96**:315, 1922.
16. Penitschka, W.: Ztschr. f. mikr.-anat. Forsch. **24**:24, 1931.
17. Palme, F.: Ztschr. f. mikr.-anat. Forsch. **36**:391, 1934.
18. Muratori, G.: Boll. Soc. ital. biol. sper. **8**:387, 1934.
19. Nonidez, J. F.: (*a*) Am. J. Anat. **57**:259, 1935; (*b*) J. Anat. **70**:215, 1936; (*c*) Anat. Rec. **69**:299, 1937.
20. Boyd, J. D.: Contrib. Embryol. **26**:1, 1937.
21. Hammond, W. S.: Am. J. Anat. **69**:265, 1941.
22. Dr. J. F. Nonidez gave aid in the identification of the aortic arch bodies.
23. de Castro, F.: Trav. du lab. de recherches biol. de l'Univ. de Madrid **24**:365, 1926.
24. Cragg, R. W.: Arch. Path. **18**:635, 1934.
25. Kohn, A.: Arch. f. mikr. Anat. u. Entwcklngsgesch. **62**:263, 1903.
26. Trinci, G., cited by Hammond.[21]
27. Busacchi, P., cited by Hammond.[21]
28. Hollinshead, W. H.: J. Comp. Neurol. **67**:133, 1937.
29. Smith, C.: Am. J. Anat. **34**:87, 1924.

found abundant chromaffin cells in the carotid body of the cow, none in that of the rat and similar cell groups in the cat. The specificity of the chromaffin reaction is questionable, as Gerard, Cordier and Lison [30] found that a variety of oxidizing salts which were not chromium compounds could produce an equally intense chromaffin reaction. De Castro [23] and others have shown that the chromaffin reaction of the carotid body in some species is due to lipoid and not to epinephrine. Furthermore, a large group of organic substances, such as hydroquinone, resorcinol, aniline, various aldehydes and ketones, and others, in addition to epinephrine, also form complex brown compounds when oxidized.[31] Hollinshead [32] has experimentally proved that the nerve endings within the aortic bodies of the cat are afferent instead of efferent. No proof has been advanced that the cells of these structures have a secretory function analogous to that of the adrenal medulla. The embryonic origin of both the carotid body and the aortic bodies will be discussed later; it suffices at this point to indicate that they do not arise from the sympathetic nervous system. Since the criteria usually considered as identifying the paraganglions do not apply to the carotid and aortic bodies, these bodies and their tumors cannot be included in the paraganglionic system.

Meijling [33] expressed the belief that the carotid body consists of ganglion cells of a special type. He used Nissl stains and Bielschowsky's silver methods and concluded that a pericellular apparatus connected the end twigs of nerve fibers with syncytially connected small peripheral autonomic ganglion cells (the glomus cells).

Goormaghtigh and Pannier [34] studied the aortic bodies, the carotid body and the abdominal aortic paraganglions of the adult cat and considered these structures as arteriovenous anastomoses. They thought each paraganglion cell had a rudimentary axis-cylinder and a cellular capsule but, unlike Meijling,[33] did not consider it a true ganglion cell as they were unable to demonstrate an intracellular neurofibrillar network. They recognized a chromaffin paraganglion related to the sympathetic system which secretes epinephrine and a nonchromaffin paraganglion related to the parasympathetic system which is truly glandular, elaborating some substance other than epinephrine. Schumacher [35] classified the carotid and aortic bodies among the arteriovenous anastomoses and expressed the belief that the specific cells are modified smooth muscle fibers that secrete acetylcholine. He considered the glomus coccygeum, the carotid body and the supracardial and abdominal bodies as being morphologically indistinguishable. The glomus coccygeum, which was first included in the paraganglionic system by Kohn,[36] was later placed by Schumacher,[37] Clara [38] and Masson [39] among the arteriovenous anastomoses, as the characteristic cell represents modified smooth muscular elements. As the coccygeal body is homologous with the cutaneous glomus, the latter is inferentially homologous with the carotid and aortic bodies if Schumacher's [37] contentions are correct. Evidence has been reviewed by Heymans

30. Gerard, P.; Cordier, R., and Lison, L.: Bull. d'histol. appliq. à la physiol. **7**:133, 1930.
31. Bennett, R. S.: Am. J. Anat. **69**:333, 1941.
32. Hollinshead, W. H.: J. Comp. Neurol. **71**:417, 1939.
33. Meijling, H. A.: Acta Neerl. morphol. **1**:193, 1938.
34. Goormaghtigh, N., and Pannier, R.: Arch. de biol., Paris **50**:455, 1939.
35. von Schumacher, S.: Ztschr. f. mikr.-anat. Forsch. **43**:107, 1938.
36. Kohn, A.: Ergebn. d. Anat. u. Entwcklngsgesch. **12**:253, 1902.
37. von Schumacher, S.: Arch. f. mikr. Anat. **71**:58, 1908.
38. Clara, M.: Ergebn. d. Anat. u. Entwcklngsgesch. **27**:246, 1927.
39. Masson, P.: Les glomus neuro-vasculaires: Histophysiologie (Policard), Paris, Hermann & Cie, 1937.

and Bouckaert,[40] Gesell,[41] Schmidt and Comroe [42] and Hollinshead [43] that the carotid and aortic bodies are chemoreceptors, as they are specialized to receive chemical stimuli indicating a fall in p_H, a rise in carbon dioxide and a decrease in oxygen of the circulating blood. Hollinshead [44] has shown that the carotid body differs from the glomus coccygeum in its vascular arrangement, in its innervation and in the structure of its cells. Nonidez [45] likewise expressed the belief that the carotid and coccygeal bodies are different structures. The evidence therefore indicates that the arteriovenous anastomoses and the chemoreceptors differ not only in morphologic character but also in function.

Hammond [21] studied the development of the aortic bodies of the cat and considered the essential cells as neuroepithelial cells that are derived mainly from vagal cells which migrate along the depressor nerves. Although the sympathetic nervous system may possibly contribute some cells, these are apparently of little importance in forming the definitive structures. The mesenchyme plays no significant role in the formation of the aortic bodies, probably contributing only to the stroma of the bodies. The derivation of the carotid body is similar, as Boyd [46] has shown that it arises from the mesenchyme of the third branchial cleft artery and from the glossopharyngeal nerve. As Hammond [21] has demonstrated, the cells of the aortic bodies are derived from the nervous system, but there is no evidence to support Meijling's [33] belief that they constitute a special type of ganglion cell.

In summation it can be stated that the carotid and aortic bodies are morphologically alike and should not be included with the paraganglions or the arteriovenous anastomoses. They have a similar origin and function as chemoreceptors. The essential cells are derived from the nervous system and can be considered as neuroepithelial sensory cells and not glandular or ganglion cells.

In view of the close similarity of the carotid and aortic bodies, a comparison of tumors of these structures may be of interest. As tumors of the carotid body have not been described in dogs, those occurring in man will be considered. The general microscopic appearance of the tumor of the carotid body placed at my disposal was somewhat similar to that of tumors of the aortic bodies, although definite differences were noted. The cellular arrangement was more alveolar, with the cells less compactly disposed. The cells showed greater variations in size, and the nuclei were usually oval or somewhat elongated. Many large hyperchromatic nuclei were likewise present, although they rarely attained the dimensions of the giant nuclei of the tumors of the aortic bodies. Peritheliomatous formations were more prominent, and a greater number of capillaries were present. There were no intravascular tumor cells, and syncytial masses were absent. Throughout the stroma and the parenchyma special stains revealed large numbers of tissue mast cells. In addition, there were many mononuclear cells that appeared to be small lymphocytes. While comparison of the tumors of the region at the base of the heart with a single tumor of a carotid body may have little significance, the morphologic evidence indicates distinct differences even though these tumors both arise from homologous structures. Whether this variation results from species differences or is due to some unknown intrinsic factor cannot be deter-

40. Heymans, C., and Bouckaert, J. J.: Ergebn. d. Physiol. **41**:28, 1939.
41. Gesell, R.: Ann. Rev. Physiol. **1**:185, 1939.
42. Schmidt, C. F., and Comroe, J. H., Jr.: Physiol. Rev. **20**:115, 1940.
43. Hollinshead, W. H.: Quart. Rev. Biol. **15**:156, 1940.
44. Hollinshead, W. H.: Anat. Rec. **84**:1, 1942.
45. Nonidez, J. F.: Anat. Rec. **82**:593, 1942.
46. Boyd, J. D.: Anat. Rec. **61**:52, 1935.

mined, although the former may be more likely. The many structural peculiarities of tumors of the carotid body in man must also be considered in the interpretation of the comparison of neoplasms derived from the carotid and aortic bodies.

Jackson's [8] observation that tumors of the region at the base of the heart resemble the infectious canine venereal sarcoma requires further comment. Briefly, the transmissible sarcoma consists of closely packed spherical or polyhedral cells with finely granular cytoplasm and a coarsely chromatic nucleus that usually contains a prominent nucleolus. Numerous mitotic figures occur. Throughout the growth are varying-sized vascular connective tissue trabeculae, which sometimes produce an alveolar structure of the tumor cells. The neoplasms differ in the following respects: In the transmissible sarcoma the cells were somewhat smaller, with giant cells absent. The cellular outlines were more distinct. The nuclei were larger in proportion to the cytoplasmic volume, and there were from two to eight mitoses in each high power field. Intravascular tumor cells were absent, and there were no syncytial masses and peritheliomatous formations. Solitary or small groups of lymphocytes occurred in scattered areas.

Although it is not within the province of this discussion to enter into the controversy on the classification of the type cell of the transmissible sarcoma, Jackson's [8] application of the term "neuroblastoma" (sympathogonioma) to the tumors of the region at the base of the heart (and consequently to the venereal tumors) appears unjustified. Immature tumors of the sympathetic nervous system, as sympathogonioma and sympathoblastoma, arise from sympathogonia and sympathoblasts, respectively.[47] The sympathogonioma is characterized by small round cells with very scanty cytoplasm that have a lymphocyte-like appearance. Pseudorosettes often occur, and delicate fibrils may be present. The sympathoblastoma consists of more mature cells with somewhat vesicular nuclei, and the cells are frequently arranged in clusters with an inner limiting membrane, forming true rosettes. In addition, axons and axon bundles may be arranged in complicated plexus formations. In morphologic characteristics the tumors of the region at the base of the heart show no resemblance to the neuroblastic tumors, and their derivation from sympathetic elements cannot be seriously considered.

Whether or not the tumors of the aortic bodies are identical with the infectious sarcoma can be settled only by transmission experiments. A noteworthy feature of the venereal tumor is the facility with which it can be transmitted to other dogs by natural or artificial means. If the tumors of the region at the base of the heart are truly venereal growths, which seems improbable from their histologic structure, the same ease of transmission should be noted. Unfortunately, this theory could not be tested, as I learned of Jackson's work after the tumors were fixed. The location of the tumor in the region of the base of the heart does not necessarily disprove that the tumor is transmissible sarcoma, as Jackson [8] specifically stated that the hypothetic first spontaneous case was one of a tumor in this location.

Although the premise that the tumors in the region at the base of the heart arise from the aortic bodies is not susceptible to direct proof, the anatomic location and the microscopic anatomy of both structures indicate their relationship. If the cellular character can be depended on as a reliable means of classifying tumors, the morphologic evidence demonstrates the histogenesis of these neoplasms. The tumor cells resembled the essential cells of the aortic bodies in their finer cytologic details and distributional patterns. The giant cells in the tumors offer no explanatory problem, as they resemble similar cells frequently seen in many cancerous and some noncancerous tumors.

47. Bielschowsky, M., in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, vol. 3, p. 1085.

In view of the embryologic and morphologic characteristics of the carotid and aortic bodies, how can tumors of these structures be interpreted? It is definitely established that the specific cells are neuroepithelial and function as chemoreceptors. On this basis the tumors of these bodies may conceivably be termed neuroepithelioma, although this designation has been already applied to specific neoplasms that occur in the retina, the spinal cord and the cerebrum which consist of cells resembling the primitive spongioblasts of the central nervous system. In histologic structure the latter neoplasms are so different from the tumors of the carotid and aortic bodies that the term "neuroepithelioma" cannot be used unless its original meaning is altered or modified by suitable adjectives. This will produce further complexities in the nomenclature, which is undesirable. Ewing [48] has summarized the different opinions concerning the nature of the tumors of the carotid body and the difficulty of devising a satisfactory designation. Although Kaufmann [49] compared the tumors of the carotid body to hamartoma, it is doubtful whether all elements of this organ participate in the tumor process. Certainly, the numerous nerve fibers of the carotid and aortic bodies are absent in the tumors of these structures. In tumors of the carotid body both the specific cells and the endothelium participate in the tumor process,[48] although in the tumors of the aortic bodies the essential cells appear to be more prominent unless the syncytial masses represent proliferated endothelium. In view of the complex structure of the carotid and aortic bodies it is suggested that neoplasms of these organs be known as tumors of the carotid body and tumors of the aortic bodies, respectively. While these designations are morphogenetically nonspecific as far as the component tissue elements of the carotid and aortic bodies are concerned, they offer no erroneous concepts relative to the histogenesis and nomenclature of the tumors. As the rich innervation of the carotid and aortic bodies is associated with their functional activity as chemoreceptors, the lack of nerve fibers in neoplasms of these structures suggests that the latter are nonfunctional.

SUMMARY

Two similar tumors occurring spontaneously in the region at the base of the heart in dogs are reported. They had essentially the same anatomic location and histologic characteristics as the aortic bodies. Therefore, it is considered, from the morphologic evidence, that the tumors originated from these structures. The aortic bodies are homologous with the carotid body, and the specific cells are neuroepithelial sensory cells that function as chemoreceptors. In view of the complex nature of the chemoreceptors it is suggested that neoplasms of these structures be designated as tumors of the aortic bodies and tumors of the carotid body.

48. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 384.

49. Kaufmann, cited by Ewing.[48]

CHRONIC GRANULOMA FOLLOWING INTRADERMAL INJECTION OF TYPHOID VACCINE

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In the spring of 1942, by order of the Military Governor of Hawaii, the entire population of the Territory [1] was immunized against smallpox and typhoid fever. An unknown but large proportion of the injections of triple typhoid vaccine given by private physicians was given as 0.1 cc. doses by the intradermal route instead of in the usual manner. The time-honored routine of one injection a week to a total of three was adhered to in nearly every instance.

Approximately 4,500 persons, most of whom were regular patients of our group, were immunized in our offices; in almost every instance the intradermal route was employed. One of us or both saw perhaps two thirds of this number. It was our observation that there was ordinarily no visible residuum from the injection at the end of a week. According to the patients' statements, the usual course followed by their reaction was that of a slight localized erythema and edema lasting from twelve to forty-eight hours and (in low incidence, variously estimated at from 2 to 10 per cent) headache, malaise or (least frequent of all) elevation of temperature. It may be further presumed that since most of these persons were our regular patients they would have returned to complain about any undesirable sequelae that might have developed following the injections.

In only 6 instances was such a sequel observed. It consisted in all cases of a peculiarly indolent granuloma-like papule at the site of injection. In 4 of the 6 cases this lesion developed at all sites of injection; in 2, however, it developed only after the third injection. Five lesions were excised in 3 of the 6 cases, and the histologic changes proved to be so remarkable and instructive that we felt them worth reporting.

CLINICAL CONSIDERATIONS

The pertinent clinical data are summarized in the accompanying table.

From the departments of clinical pathology and dermatology of the Clinic.

1. Excepted are those persons already immunized in 1942, or certified by their physicians as too young, too old or too ill to tolerate the procedure.

In no instance was there a history of a more than ordinarily severe or painful reaction to the injection of the vaccine. In each case the patient stated that a red papule developed at the site of injection within a day or two, and thereafter merely persisted, almost unchanged. In 2 instances (cases 3 and 5) secondary infection of the site, with formation of an abscess, was suspected; the lesions were incised and a little cloudy fluid expressed, which on culture in 1 case proved sterile.

Clinical Data Regarding Six Patients in Whom Chronic Granuloma Followed Intradermal Injections of Triple Typhoid Vaccine

Patient	Race	Age	Sex	Date of Injections; Previous Injections	Clinical Appearance and Course
1	White	35	M	February 1942; previously vaccinated on four occasions, last in 1937, with severe reactions each time	Small shotty red nodules at each site of injection, the largest following the third injection. One excised at 3 months; others slowly involuted and were gone in 8 months
2	Portuguese	43	M	April 1, 7 and 14, 1942	Only the third injection resulted in a nodule which was dull red and elevated; excised 5 weeks after vaccination
3	White	46	F	Feb. 28, March 7 and 14, 1942; no previous vaccination	Indolent dull red papules at each site of injection. Two incised in April and pus obtained; cultures sterile. All three excised at 6½ months
4	Chinese-Hawaiian	13	F	Feb. 28, March 7 and 14, 1942; no previous vaccination	Dull red, indolent, elevated papules at each site of injection (fig. *A*). No involution apparent at 6½ months
5	Chinese	30	M	March 1942; previous vaccination by subcutaneous route in 1933	Only the third injection resulted in an "abscess," which was incised and evacuated in April. It remained as a dull red nodule with no change up to the time of its last observation in October. No sequelae to the first two injections
6	Japanese	23	F	March 12, 19 and 26, 1942; no previous vaccination	Small shotty red raised nodules at each site of injection. All lesions healed spontaneously in August, 5 months after the injections. No visible residuum in September

All three lesions in case 6 and the two unexcised lesions in case 1 persisted without change for about five months and then rapidly involuted and disappeared within a week or two. The lesions in cases 4 and 5 had not begun to involute at the time of this report, some seven months after the period of the injections.

The lesions in all cases when first observed consisted of rubbery-firm elevated lenticular papules between 4 and 12 mm. in diameter, round or nearly so, and dull red or bluish red, with occasional faint peripheral hyperpigmentation. The overlying epidermis was firmly fixed to them. They were neither tender nor pruritic. In cases 3 and 5 there was slight central pallor and softening, apparently indicative of necrosis which had progressed to the point of liquefaction.

In no instance did the lesions suggest a furuncle or any other variety of pyoderma except, of course, for the transient suspicion of abscess formation in the two last-mentioned cases. The appearance was uniformly that of a chronic granuloma except for the extreme indolence, which suggested neoplasia: fibroma, for example. It was this feature that made us almost certain at first that we were dealing with histiocytoma cutis. This expectation was dispelled by the first biopsy.

HISTOLOGIC DESCRIPTIONS

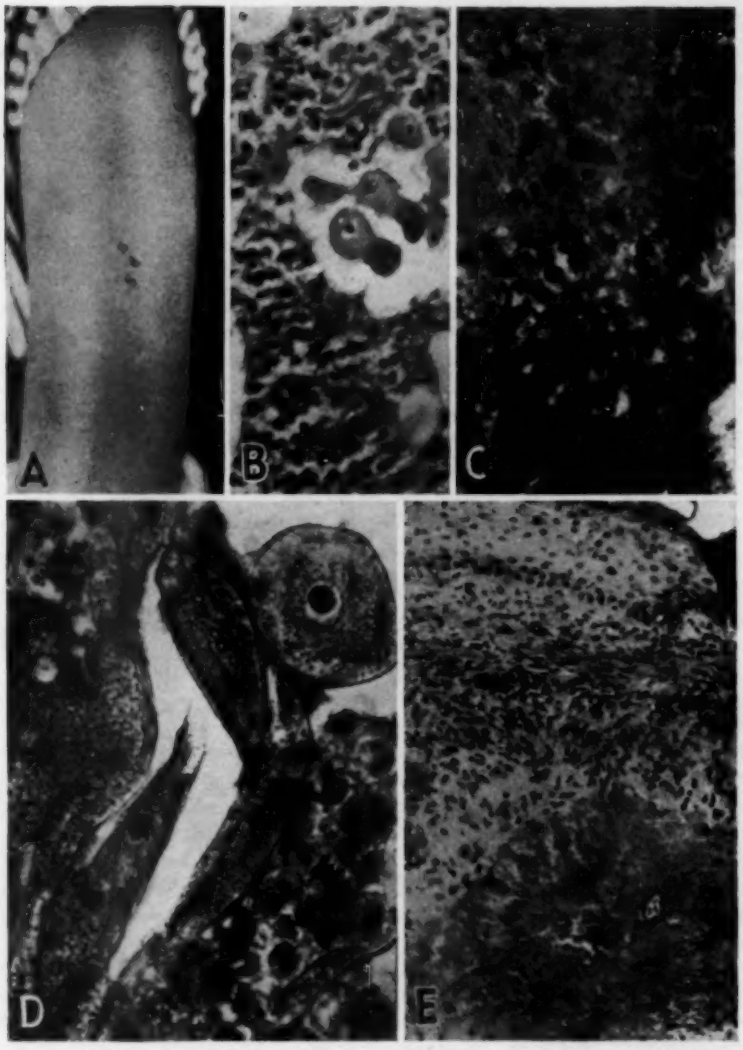
CASE 1.—The lesion was three months old. The epidermis was normal. The papillary portion of the corium was edematous, as manifested by widened spaces between the collagen bundles. About 0.5 mm. beneath the epidermis was a poorly circumscribed spherical lesion composed centrally of degenerating collagen bundles and peripherally of dense accumulations of lymphocytes. The latter were arranged predominantly about blood vessels and sweat glands. The lesion extended down to the subcutaneous fat but involved this layer only slightly. A few histiocytes were present, most numerous in the central area of necrobiosis but also present at the periphery, particularly in the form of poorly defined small giant cells. Droplets of sudanophilic lipoid material were demonstrated in some of these cells by fat stains.

CASE 2.—The lesion was five weeks old and measured about 3 by 6 mm. It occupied the most superficial portion of the corium, immediately beneath the epidermis, with its long axis parallel to the surface. It was partly enclosed on one side and toward the surface by a stratified squamous epithelial layer, which was distinct from the covering epidermis, suggesting that the process might have started within the pilosebaceous apparatus.

The lesion itself consisted of a large necrotic center composed of fibrinous material infiltrated by polymorphonuclear leukocytes, surrounded by a rim of perpendicularly arranged reticuloendothelial cells and, outside these, dense accumulations of lymphocytes. Several aggregations of giant cells of both Langhans and foreign body types were observed further toward the periphery. The lymphocytes were grouped predominantly about blood vessels and sweat glands. Many sharply outlined, extremely large, swollen histiocytes, approximating foam cells in structure, were present within the necrotic center (*B* in fig.). Fibrosis was minimal; the reaction was primarily histiocytic in nature.

Frozen sections stained with sudan III and hematoxylin revealed an interesting picture. The perpendicularly arranged reticuloendothelial cells at the periphery contained, almost without exception, abundant lipoid droplets (*C* in fig.). The same was true of the greatly swollen histiocytes (foam cells) in the necrotic center (*D* in fig.). Much extracellular lipoid material was also present, particularly near the margin of the necrotic central portion of the lesion.

CASE 3.—The lesions were six and a half months old. Lesion 1 was spherical, about 4 mm. in diameter, and consisted of a conglomeration of small granulomas, each composed of a necrotic center and a peripheral perpendicularly arranged rim of reticuloendothelial cells (*E* in fig.). The latter contained abundant lipoid material. In this lesion, in contrast with those in cases 1 and 2, moderate fibroblastic proliferation was observed, surrounding the lesion and tending to wall it off. There were also the usual dense foci of lymphocytes, arranged as before, about the vessels and the sweat glands. Many of the smaller arterioles presented thickened



A, typical lesions of six and one half months' duration (case 4).

B, portion of the necrotic center of a lesion in case 2, showing numerous polymorphonuclear leukocytes and large swollen histiocytes approximating foam cells in morphologic character. Hematoxylin and eosin; × 250.

C, same lesion as *B*, showing peripheral zone of reticuloendothelial cells stained orange (black in the photograph). Sudan III and hematoxylin; × 250.

D, same lesion as in *B* and *C*, showing many spindle-shaped histiocytes and one swollen foam cell (upper right), all filled by fine lipoid droplets (black in the photograph). Sudan III and hematoxylin; × 600.

E, lesion 1 in case 3, typical of all the typhoid granulomas. Note the necrotic center, the zone of reticuloendothelial cells around it and the peripheral aggregations of lymphocytes. Hematoxylin and eosin; × 130.

walls, and the lumens of some were completely obliterated, a feature not observed in the lesions previously studied. Several giant cells of the Langhans type were found at the periphery of many of the smaller granulomas.

The epidermis covering lesion 2 was moderately acanthotic. There were two partially confluent, roughly spherical granulomas, consisting of necrotic centers, measuring together some 6 mm. in diameter and extending down to the subcutaneous fat. Dense perivascular accumulations of lymphocytes were again present, as was the surrounding rim of fat-filled reticuloendothelial cells.

Lesion 3 consisted of a single large spherical granuloma, about 5 mm. in diameter. Ulceration of the epidermis had occurred over its most superficial portion. The lesion consisted of the usual necrotic center and rim of lipoid-filled reticuloendothelial cells. Many small satellite granulomas, composed largely of histiocytes and Langhans giant cells, were present at the margin.

COMMENT

The tissue response to intradermal typhoid vaccine in all the lesions studied was predominantly reticuloendothelial in nature. In this respect at least the lesions were similar to those produced by living typhoid bacilli. It has long been known that the typhoid bacillus incites a characteristic "monocytic" tissue reaction distinguished by a peculiar lack of suppuration and the appearance of large spherical macrophages. It is generally believed that the latter cells are members of the reticuloendothelial system; the fact that they often contain cellular debris and other foreign material lends support to this belief. The tissue reaction in typhoid fever is similar to that produced by the tubercle bacillus, but more diffuse.

The lesions we have observed following intradermal injections of typhoid vaccine were all well circumscribed, and were in every sense true chronic granulomas. They were histologically quite similar to the lesions of tuberculosis, leprosy, and certain other of the chronic specific granulomas. The type of necrosis observed in them, however, differs from the caseation necrosis of tuberculosis. The material in the broken-down centers of the lesions appears homogeneously necrotic and is to some extent infiltrated by polymorphonuclear leukocytes.

Many swollen macrophages approximating foam cells in morphologic character and similar to the "monocytes" of typhoid fever were present in the necrotic center of the lesion removed in case 2 (*B* in fig.). All of them contained more or less lipoid material in the form of tiny droplets. Many exhibited evidence of degeneration. Various transitional stages between spindle-shaped lipoid-laden histiocytes and swollen foam cells could be noted; this transition is particularly well shown in *D* in the figure. In each lesion the phagocytic—reticuloendothelial—component of the granuloma was well outlined by sudan III stain. Without such stains the spindle-shaped reticuloendothelial cells might easily be mistaken for fibroblasts (*D* in fig.); this error would of course never be made in the case of the adjacent swollen foam cells.

This finding is not peculiar to typhoid granuloma; the epithelioid cells of the tubercle and the reticuloendothelial cells of other chronic specific granulomas often contain demonstrable lipoid material. The same statement holds true for the reticuloendothelial cells present in nonspecific inflammatory reactions. The presence of lipoid within the cells of this system is simply a manifestation of their phagocytic ability and hence, in our opinion, of their reticuloendothelial origin. We believe that such intracellular lipoid material is not usually present in fibroblasts and that lipoid stains are therefore singularly useful in differentiating these two cell systems. We have amplified this view, which is a somewhat controversial one, elsewhere.[2]

SUMMARY

A peculiar, hitherto undescribed focal granulomatous reaction to intradermal injections of triple typhoid vaccine has been observed by us in 6 patients. The vaccine employed was in all cases prepared by the United States Army, using the Boxill strain. No correlation appears to exist between the occurrence of this reaction and a history of previous injections of typhoid vaccine. It is not known whether the occurrence of this reaction vitiates the effect of the vaccination or not. The chief practical importance of the reaction would appear to lie in the possible cosmetic consequences, should the vaccination be performed in a conspicuous location. The observation is of theoretic importance because it so clearly demonstrates the similarity between the histiocytic response to injections of triple typhoid vaccine and the histiocytic response to virulent typhoid bacilli and because it constitutes another link in the growing chain of evidence that spindle-shaped cells in granulation tissue may be histiocytes and not, as is so often and so casually assumed, fibroblasts.

2. Arnold, H. L., Jr., and Tilden, I. L.: Arch. Dermat. & Syph. **47**:498, 1943.

PATHOLOGIC CHANGES INDUCED IN VARIOUS SPECIES BY OVERDOSAGE WITH DESOXYCORTICOSTERONE

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The ever increasing use in clinical medicine of desoxycorticosterone and other corticoid steroids [1] makes it rather important to elucidate as far as possible the various effects of such therapy. For this reason a good deal of attention has been given in recent years to such preparations from the standpoint of toxicology, and it is the object of this communication to report additional observations concerning the pathologic changes induced by overdosage with desoxycorticosterone acetate in various species. Special attention will be given to the ability of this substance to sensitize the body to the toxic actions of sodium chloride.

It is outside the scope of the present report to review the relevant literature completely, but a brief survey of the salient toxicologic facts concerning desoxycorticosterone acetate and sodium chloride will facilitate the evaluation of our own observations. Although it has been known for some time that insufficiency of adrenal cortex decreases the chloride concentration of the blood and that suitable extracts of adrenal cortex restore the concentration to normal, a disturbance in the chloride metabolism of intact animals could not be produced by overdosage with desoxycorticosterone acetate until large amounts of it became available. At this time the surprising fact was established that in the rat overdosage with desoxycorticosterone acetate causes hypochloremia similar to that seen in adrenal insufficiency.[1a] Concomitantly the chloride concentration in the muscles tends to rise above normal.[2] Apparently unaware of these findings, Ferrebee and associates [3] reported quite independently that in dogs the chloride and especially the sodium concentration of muscles tends to rise following prolonged administration of desoxycorticosterone acetate so that there appears to be no doubt concerning the ability of this steroid to induce profound changes in the metabolism of sodium chloride.

Another interesting observation is that prolonged administration of large doses of desoxycorticosterone acetate causes motor disturbances in dogs. These disturbances are characterized by periodic spells of paralysis. Kuhlmann and

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Dr. Erwin Schwenk, of the Schering Corporation, of Bloomfield, N. J., supplied the desoxycorticosterone acetate necessary for these experiments.

1. The term "corticoid" is used to describe substances having an action resembling that of the adrenal cortex (Nature, London **148**:84, 1941).

1a. Selye, H., and Dosne, C.: Proc. Soc. Exper. Biol. & Med. **44**:165, 1940.

2. Selye, H., and Dosne, C.: Am. J. Physiol. **132**:522, 1941.

3. Ferrebee, J. W.; Parker, D.; Carnes, W. H.; Gerity, M. K.; Atchley, D. W., and Loeb, R. F.: Am. J. Physiol. **135**:230, 1941.

co-workers,[4] who first described this effect, compared these spells with those occurring in familial periodic paralysis in man. The paralysis is accompanied by a decrease of the potassium in serum and muscle and has generally been considered to be a purely muscular defect due to a disturbance in the electrolytes of muscle.[5] The similarity between familial periodic paralysis in man and these symptoms of overdosage was further emphasized by the observation that serum potassium is usually low and that administration of potassium causes disappearance of the motor disturbances in both these conditions.[6] The assumption that the deficiency of serum potassium in itself is the cause of the paralysis is refuted by Butler and co-workers,[7] who found that such compounds as testosterone propionate and methyl testosterone can elicit an even more significant fall in serum potassium without any sign of paralysis.

It has also been noted that overdosage with desoxycorticosterone acetate may lead to marked polyuria in the dog,[8] the rat[9] and man.[5b] This diuretic potency is also demonstrable in progesterone (which, like desoxycorticosterone, maintains the life of adrenalectomized animals) but not in steroids which are devoid of corticoid actions simulating those of adrenal cortex. The diuresis is especially prominent in hypophysectomized rats, and therefore it is reasonable to assume that the pituitary gland tends to counteract this effect.[10]

Although Ragan and associates[5] were unable to detect any renal changes in their dogs in which paralysis developed as a result of overdosage with desoxycorticosterone acetate, it is known that this steroid has a distinct renotropic action. This effectiveness is shared by progesterone, and combined treatment with the two steroids results in a more pronounced enlargement of the kidneys than can be produced by either alone.[11] The effect is not mediated through the pituitary gland, as it is quite evident even after hypophysectomy.[12] Histologically, the renal changes so far produced in mammals have been qualitatively similar to those elicited by the quantitatively more potent testosterone[13]; that is to say, the cells of the parietal layer of Bowman's capsule are slightly hypertrophied, but otherwise the glomerulus remains intact, while the rest of the nephron, especially the proximal and the distal convoluted tubules, undergoes marked hypertrophy. More recently it was found, however, that in domestic fowl typical nephrosclerosis with hyalinization of the capillaries of the glomerular tuft can readily be induced with desoxycorticosterone acetate.[14] The renal changes are accompanied by cardiovascular lesions similar to those seen in hypertensive heart disease of man.

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4. Kuhlmann, D.; Ragan, C.; Ferrebee, J. W.; Atchley, D. W., and Loeb, R. F.: Science **90**:496, 1939.

5. (*a*) Ragan, C.; Ferrebee, J. W.; Phyfe, P.; Atchley, D. W., and Loeb, R. F.: Am. J. Physiol. **131**:73, 1940. (*b*) Moehlig, R. C., and Jaffe, L.: J. Lab. & Clin. Med. **27**:1009, 1942. Ferrebee and others.[3]

6. Pudenz, R. H.; McIntosh, J. F., and McEachern, D.: J. A. M. A. **111**:2253, 1938. Ferrebee and others.[3]

7. Butler, A. M.; Talbot, N. B., and MacLachlan, E. A.: Proc. Soc. Exper. Biol. & Med. **51**:378, 1942.

8. Mulinos, M. G.; Spingarn, C. L., and Lojkin, M. E.: Am. J. Physiol. **135**:102, 1942. Kuhlmann and others.[4] Moehlig and Jaffe.[5b]

9. Selye, H., and Bassett, L.: Proc. Soc. Exper. Biol. & Med. **45**:272, 1940.

10. Selye, H., and Bassett, L.: Proc. Soc. Exper. Biol. & Med. **44**:502, 1940; footnote 9.

11. Selye, H.: Canad M. A. J. **42**:113, 1940.

12. Selye, H.: J. Urol. **46**:110, 1941.

13. Selye, H.: J. Urol. **42**:637, 1939; J. Endocrinol. **1**:208, 1939.

14. Selye, H.: Canad. M. A. J. **47**:515, 1942.

The antiuremic action of desoxycorticosterone acetate was demonstrated by the fact that rats pretreated with this steroid survive subsequent nephrectomy longer than controls.[15] This observation has been confirmed by various investigators,[16] but since no antiuremic effect is obtained if treatment is started after ablation of the kidneys, it has been assumed that the beneficial action is due to the fact that overdosage with desoxycorticosterone acetate tends to deplete the body of potassium, thus inhibiting the tendency of this electrolyte to reach toxic concentrations in the blood of subsequently nephrectomized animals. The aforementioned observations of Butler and co-workers [7] which indicate that testosterone elicits a similar depletion of body potassium are not in agreement with this interpretation, since testoid steroids are devoid of antiuremic effects.

Other interesting results of overdosage with desoxycorticosterone acetate are atrophy of the thymus [17] and compensatory atrophy of the adrenal cortex. Curiously, following long periods of treatment, the adrenal medulla may also show certain signs of involution.[18]

Finally it should be mentioned that if the organism of the rat is suddenly flooded with desoxycorticosterone acetate (as after intravenous or intraperitoneal administration of large doses) general surgical anesthesia ensues.[19] It is somewhat reminiscent of the paralytic seizures mentioned, since in both conditions one is dealing with motor disturbances. The anesthetic effect has always been attributed to an action on the central nervous system since during it direct stimulation of motor nerves is still capable of eliciting muscular contractions. Similar experiments have not been performed in connection with the periodic paralysis caused by chronic overdosage with desoxycorticosterone acetate. However, it has been assumed that in the latter condition the muscles are at fault because of the derangement in their electrolyte content.

The symptoms of overdosage with sodium chloride resemble those produced by toxic doses of desoxycorticosterone acetate inasmuch as an excess of sodium chloride causes cardiac dilatation, more or less characteristic renal changes and various types of motor disturbances.[20] In this connection it is of special interest to note that fowl, which are particularly sensitive to desoxycorticosterone acetate, are also unusually sensitive to overdosage with sodium chloride and respond with marked nephrosclerosis, cardiac dilatation and edema when overdosed with either of these two compounds.[21]

Ragan and co-workers [5a] stated that in dogs, even if large doses of sodium chloride are given in combination with desoxycorticosterone acetate, tissue edema

15. Selye, H.: Canad. M. A. J. **43**:333, 1940.

16. Selye, H., and Nielsen, K.: Proc. Soc. Exper. Biol. & Med. **46**:541, 1941. Dosne, C.: Am. J. Physiol. **134**:71, 1941. Rodbard, S.: Federation Proc. **1**:73, 1942. Selye, H.: Actas y trabajos II Congreso panamericano de endocrinologia, Montevideo, March 6-8, 1941. Durlacher, S. H., and Darrow, D. C.: Am. J. Physiol. **136**:577, 1942. Winkler, A. W.; Smith, P. K., and Hoff, H. E.: J. Clin. Investigation **21**:419, 1942.

17. Selye, H.: J. Anat. **76**:94, 1941.

18. Selye, H.: J. A. M. A. **115**:2246 (Dec. 28) 1940; footnote 1.

19. Selye, H.: Proc. Soc. Exper. Biol. & Med. **46**:116, 1941; Endocrinology **30**:437, 1942.

20. Falck: Virchows Arch. f. path. Anat. **56**:315, 1892. Richet, C.: Compt. rend. Soc. de biol. **34**:363, 1882. Cohnheim, J., and Lichtheim, L.: Virchows Arch. f. path. Anat. **69**: 106, 1877. Raum, J.: Arch. f. exper. Path. u. Pharmakol. **29**:353, 1892. Leopold, E. J.: Ztschr. f. klin. Med. **60**:490, 1906. Rössle, R.: Berl. klin. Wchnschr. **45**:1165, 1907. Hoessli, H.: Frankfurt. Ztschr. f. Path. **4**:258, 1910. La Grutta, L.: Riv. di pat. sper. **1**:241, 1934. Cutting, R. A.; Larson, P. S., and Lands, A. M.: Arch. Surg. **38**:599, 1939. Kleiner, I. S., and Dotti, L. B.: New York M. Coll. & Flower Hosp. Bull. **3**:309, 1940.

21. Selye, H., and Stone, H.: Proc. Soc. Exper. Biol. & Med., to be published.

or congestive heart failure cannot be produced. Altschule and Zamcheck,[22] however, described the case of a man in whom marked anasarca developed, with effusions into the body cavities, albuminuria and congestive heart failure as a result of simultaneous treatment with comparatively large doses of sodium chloride and an extract of adrenal cortex. Our observations in fowl likewise indicate a summation and perhaps even a potentiation of the pharmacologic effects of these two agents if they are given simultaneously.[21] Therefore, we decided to undertake a detailed study of the symptoms of overdosage with desoxycorticosterone acetate in various mammals such as the dog, the monkey and the rat, since it appeared rather likely that these species, which are comparatively resistant to desoxycorticosterone acetate, could be sensitized with sodium chloride to the actions of this steroid. Such an investigation appeared to be particularly important since the experiments on fowl had revealed a correlation between nephrosclerosis and the adrenal cortex, and it was hoped that systematic studies along these lines might reveal further leads concerning the cause of this common and frequently fatal disease of man.

MANIFESTATIONS OF OVERDOSAGE WITH DESOXYCORTICOSTERONE ACETATE IN THE DOG, THE MONKEY AND THE RAT

Dog.—Four recently weaned puppies, weighing 900 to 1,300 Gm., were used for this experiment. According to the dealer, they were 1 month old at the initiation of treatment. All belonged to a single litter, and although they were not pure bred, they were of almost identical appearance at the beginning of treatment. The males were about 200 Gm. heavier than the females, but the average weight of the male and the female used as controls and that of the male and the female treated with desoxycorticosterone acetate were identical. The two treated pups received 5 mg. of this steroid in 0.1 cc. of peanut oil subcutaneously twice daily for a period of one week, after which the daily dose was doubled. By the end of the second week of treatment the dose was raised to 20 mg. of desoxycorticosterone acetate in 0.4 cc. of peanut oil, given subcutaneously twice daily. This dose level was maintained during the remainder of the experimental period. It will be noted that desoxycorticosterone acetate is not soluble in oil in such a high concentration; hence it had to be administered in the form of a suspension of fine crystals. The delayed absorption of the crystals from the suspension increases their hormonal effectiveness. In order to obtain good absorption, the site of the injection was varied over almost the entire cutaneous area, and inspection at autopsy showed that removal of the crystals from the subcutaneous tissue was practically complete. The diet of these animals consisted of Purina dog chow checkers [22a] and milk. During the first stages of the experiment the treated animals ate and drank much more than the controls and exhibited a more rapid increase of weight in spite of the fact that polyuria like that of diabetes insipidus developed. Later, however, the increase of weight was greater in the controls.

During the first month of treatment no signs of paralysis were detected. At this time 1 per cent sodium chloride solution was added to the milk for both the experimental and the control group. Forty-eight hours later marked paralysis, especially of the neck and shoulder muscles, developed in both dogs treated with desoxycorticosterone acetate. It did not appear to influence their well-being otherwise, since they continued to eat and drink and showed obvious signs of pleasure, such as wagging the tail and jumping about, when approached by their caretaker. The characteristic posture of these animals was to stand with the head bent downward between the shoulders but with the body otherwise erect, and they were quite able to run about (fig. 1). Slow intravenous injection of 20 cc. of 2 per cent potassium chloride solution did not effect any dramatic recovery at this time, but on substitution of the salted milk by ordinary milk the animals recovered within two days. Ten days after this episode they were again forced to drink 1 per cent saline milk and again showed muscular weakness within forty-eight hours. This time the muscles of the legs were also involved, and both dogs, especially the female, became comatose. We could

22. Altschule, M. D., and Zamcheck, N.: J. Clin. Endocrinol. **2**:269, 1942.

22a. Purina dog chow checkers contain meat meal, dried skim milk, prepared cereals and vegetable roughage fortified with minerals and vitamins.

not establish whether the symptoms of apparent stupor were indicative of an involvement of the central nervous system or were due merely to generalized muscular weakness. When the dogs were given plain milk, recovery was apparently again complete in forty-eight hours. Three days later the 2 male dogs (experimental and control) received an intravenous infusion of 40 cc. of a 5 per cent sodium chloride solution, administered within a period of one hour. While the control dog showed no indications of any ill effects, the animal treated with desoxycorticosterone acetate exhibited obvious signs of muscular weakness as well as spells of vomiting and diarrhea. Again recovery was rapid, in spite of continued treatment with desoxycorticosterone acetate. Two days later a similar experiment was performed on the 2 female dogs, but this time 100 cc. of a 5 per cent saline solution were given in four intravenous injections over a period of two hours. At the end of the period of injection vomiting occurred both in the control and in the treated animal, but while the former rapidly recovered, the latter showed marked muscular weakness and tremor without any tendency toward recovery. Six hours after the injection the treated animal was in deep coma, which resembled general anesthesia. At this time 20 cc. of a 2 per cent solution of potassium chloride was administered intravenously without any beneficial effect, and shortly afterward the animal died. The total length of observation in the case of this bitch was forty-seven days, and it will be noted that, although the daily treatment with desoxycorticosterone acetate was continuous, paralytic seizures were observed

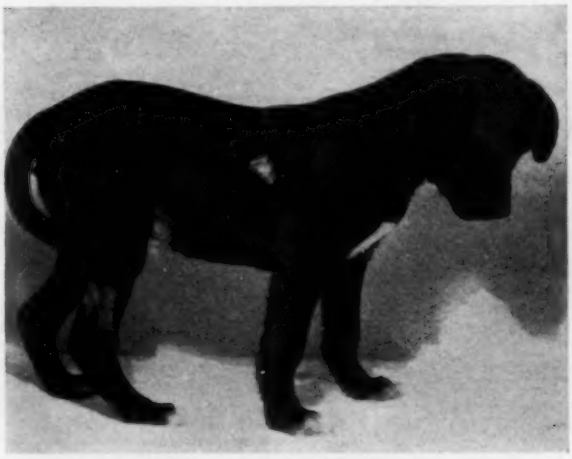

Fig. 1.—A male dog treated with desoxycorticosterone acetate and then given sodium chloride. The posture is typical of beginning paralysis of the muscles of the neck. In later stages all skeletal muscle groups were paralyzed, so that the animal was unable to stand on its feet.

only when sodium chloride was administered orally or intravenously. Conversely, whenever sodium chloride was given, an attack of paralysis ensued. The control female was killed at the time the steroid-treated bitch died. The animals were dissected, and the report of the gross as well as of the microscopic observations will be given later.

The remaining 2 male dogs were kept without addition of saline solution to their diet until the sixty-second day of treatment with desoxycorticosterone acetate. Then 1 per cent sodium chloride solution was again added to their milk. Again paralysis was elicited only in the steroid-treated animal, and the symptoms disappeared after withdrawal of the salt. On the seventieth day 2 per cent sodium chloride solution was added to the milk, and while the control animal readily tolerated even this high concentration, the steroid-treated dog showed progressive paralysis, beginning at the neck and shoulders and gradually involving practically all the muscles of the body. Approximately thirty hours after the initiation of this salt treatment the experimental animal died. At this time the control male was killed for comparison.

In summarizing the clinical features of this experiment we may say that even the enormous doses of desoxycorticosterone acetate given to these small puppies failed to cause any dramatic clinical symptoms as long as the intake of sodium chloride was kept low. It is rather noteworthy that although a total amount of 1,530 mg. was administered to the female during

the forty-seven days of treatment and 2,450 mg. to the male over seventy days, which at the time of death weighed only 2.6 and 4.25 Kg., respectively, no signs of overdosage appeared except during periods of high intake of sodium chloride. In comparison with the doses used for the human being, which are usually kept at a level of about 0.1 mg. per kilogram per day, the enormity of the doses administered to our dogs is evident, since they amounted to approximately 10 mg. per kilogram per day. Therefore the damaging actions noted in our experiments should not deter the clinician from employing desoxycorticosterone acetate in the usual doses whenever this treatment is indicated. However, there is no reason to believe that under abnormal conditions cortical secretion in man could not reach the fantastically high levels realized in our animals. Should such excess be reached, it might constitute an endogenous overdosage of similar severity.

It is particularly noteworthy that neither during life nor at autopsy did we note any edema of tissues. The only indication of this observed was the development of large abscesses at the sites of the subcutaneous injections in the treated dogs at the time that sodium chloride was being administered. Curiously, such abscesses invariably retrogressed when the salt was withdrawn.

The most important organs were weighed after dissection, and the weights are summarized in the accompanying table.

Weights of Organs of Dogs Treated with Desoxycorticosterone Acetate Compared with Those of the Corresponding Organs of Control Dogs

			Organ Weights									
Sex*	Treatment	Body Weight, Kg.	Kidney, Gm.	Heart, Gm.	Liver, Gm.	Spleen, Gm.	Pancreas, Gm.	Thyroid Gland, Mg.	Adrenal Gland, Mg.	Thymus, Gm.	Pituitary Gland, Mg.	Testis, Mg.
Female	Desoxycorticosterone acetate	2.6	27.5	23.0	116.5	6.9	6.6	373	92	2.4	31.5	
Male	Desoxycorticosterone acetate	4.25	50.5	36.5	225.0	6.1	10.0	541	348	5.4	54.0	320
Female	Control	2.8	19.2	17.5	119.0	4.5	5.9	256.1	255	4.9	29.0	
Male	Control	5.3	34.0	35.5	170.0	7.5	12.0	556	610	10.0	44.5	768

* It should be kept in mind that each experimental animal must be compared with the control of the same sex, especially since the females were killed at an earlier age than the males.

At autopsy, both in the male and in the female dog treated with desoxycorticosterone acetate the most conspicuous change was in the kidneys, which were greatly enlarged and very pale. The surfaces were slightly irregular, and the organs exhibited the appearance of the "large white kidney." Histologic examination revealed that, especially in the male, in which treatment was more prolonged, the diameter of the convoluted tubules (both proximal and distal) was increased, owing to cellular hypertrophy and dilatation of the lumen. In many tubules the lumen was obstructed by hyaline casts, and desquamation of cells into the lumen was quite common. Many of the glomeruli contained numerous large dark cells, especially in the vicinity of the hilus. Similar epithelioid cells were observed in the connective tissue surrounding the glomeruli (fig. 2 *A* and *B*). The walls of the afferent glomerular arterioles were likewise thickened, but the large renal vessels showed no conspicuous abnormality.

The heart was also enlarged and pale in the treated animals, although the increase was not as obvious in the male as it was in the female. It must be kept in mind, however, that although the increase in the actual weight of the heart was not striking, the weight as a percentage of body weight was far greater in the treated animals. Sections stained with sudan III revealed that the pallor of the heart was not due to accumulation of fat. It may be mentioned at this time that the skeletal muscles in the treated animals also appeared somewhat pale when compared with those of the controls. A section of the heart muscle stained with hematoxylin and eosin revealed no changes except a slight degree of muscular hypertrophy and a trace of edema in the stroma.

The blood vessels of the treated animals revealed no definite sign of sclerosis, although in general the arteries appeared somewhat thickened. In the arcus aortae, inspection with

the naked eye revealed an irregular yellowish spot which was just detectable in the female but quite marked in the more chronically treated male. Histologically, this region was characterized by irregularities in the elastic membranes between the muscle layers. In many places the elastic fibers were swollen and in the process of degeneration (fig. 2 *C*).

The adrenal glands of the treated animals revealed an extreme degree of compensatory atrophy. Histologically, all layers of the cortex participated in this involution. In addition

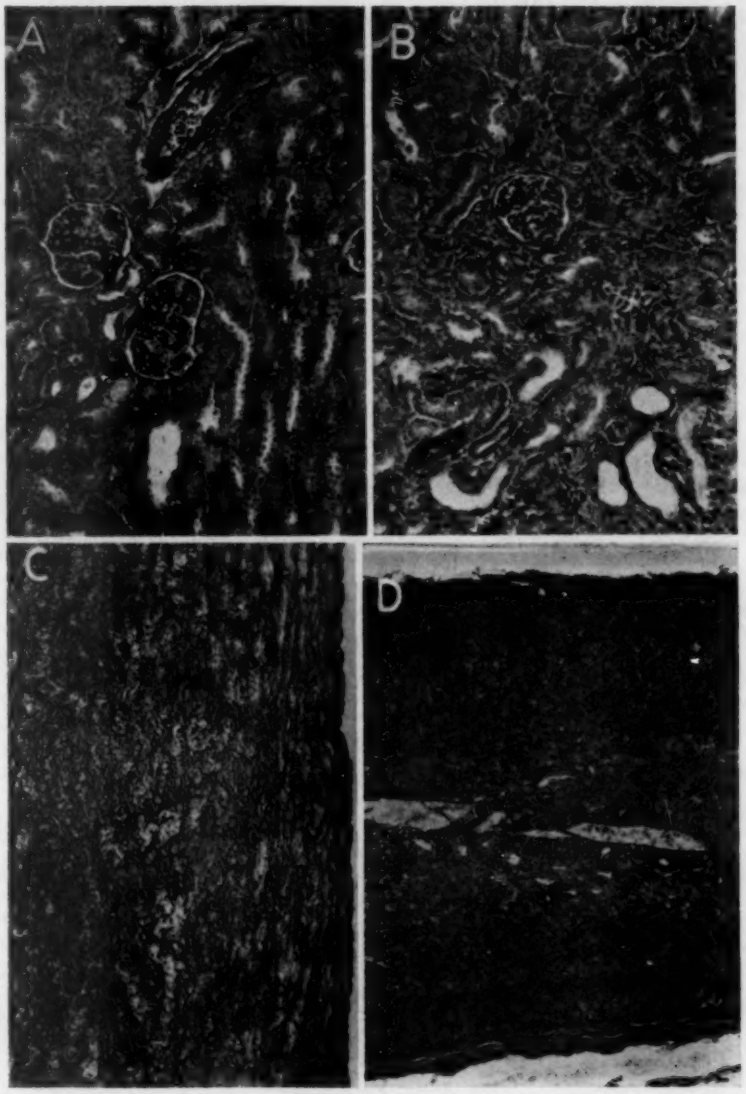

Fig. 2.—*A* and *B* are sections through a kidney of a female dog treated with desoxycorticosterone acetate showing hypertrophy of cells of convoluted tubules and varying degrees of glomerular changes. *A* shows the dark cells in the glomerulus, especially in its hilus; *B* shows thickening of the glomerular basement membrane. *C* is a cross section through the aortic arch of a male dog treated with desoxycorticosterone acetate, showing swelling and beginning degeneration of elastic tissue. *D* is a cross section through the center of the left adrenal gland of a male dog treated with desoxycorticosterone acetate, showing reduction in size of both the cortex and the medulla.

to the cortical atrophy there was marked leukocytic infiltration of the cortex similar to that seen in cases of so-called primary Addison's disease. The medullary cells were like-

wise atrophic, as a result of which the total diameter of the medulla was greatly reduced (fig. 2 *D* and 3 *A*). The fact that the medulla may participate in the compensatory atrophy of the adrenal gland after administration of desoxycorticosterone acetate has been demonstrated in rats,[18] but in the latter species it was never as pronounced as in the dogs of the present series.

The thymus likewise showed involution, confirming previous observations recorded in the introductory section of this paper. The long bones of the treated animals were shorter but broader than those of the controls, and the joint cartilages were thickened. The liver, the

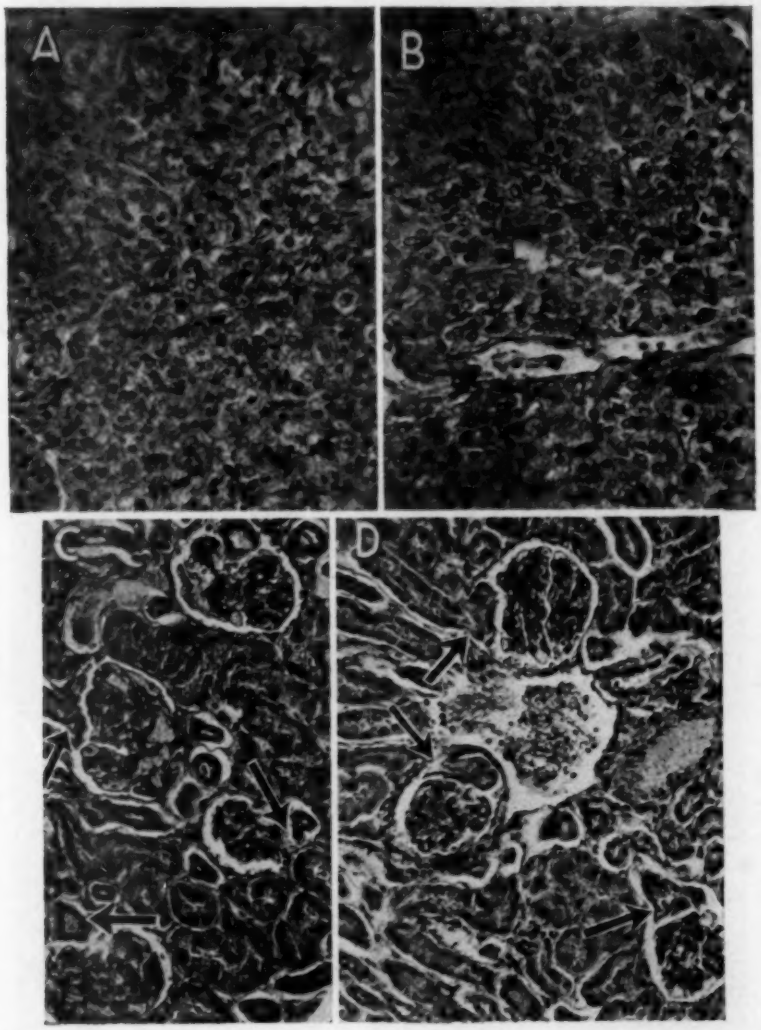

Fig. 3.—*A* shows marked lymphocytic infiltration of the atrophic zona fasciculata of the adrenal cortex of a male dog treated with desoxycorticosterone acetate. *B* shows atrophic adrenal medulla from a treated male dog. *C* and *D* represent various types of invagination of proximal convoluted tubule segments between glomerular capillaries and the parietal lamina of Bowman's capsule (points of invagination marked by arrows) in a monkey treated with desoxycorticosterone acetate.

spleen, the pancreas and the thyroid, parathyroid and pituitary glands showed no very pronounced changes either in total weight or in microscopic structure.

At the time of death blood samples were taken from the heart, of the 2 male dogs for determination of the chloride and nonprotein nitrogen contents. The chloride content of

the plasma expressed as milligrams of sodium chloride per hundred cubic centimeters was 650 mg. in the control and 710 mg. in the treated animal. Since these determinations were made during a period of excessive intake of sodium chloride, they may be taken as additional evidence of impaired tolerance for this salt. They do not indicate, however, that on a normal intake dogs are unable to respond with the marked hypochloremia commonly seen in rats treated with desoxycorticosterone acetate. The plasma nonprotein nitrogen amounted to 37 mg. per hundred cubic centimeters in the control and to 62 mg. per hundred cubic centimeters in the treated dog, suggesting a definite impairment in the renal excretion of nitrogenous end products.

Monkey.—Two male rhesus monkeys (Macacca mulatta), weighing 2,900 and 2,950 Gm. at the beginning of the experiment, were used to study the effects of severe overdosage with desoxycorticosterone acetate in the primate. One was used as a control, while the other received 20 mg. of the steroid suspended in 0.4 cc. of peanut oil twice daily by subcutaneous injection. Treatment was continued for sixty-three days. The animals were fed Purina dog chow, carrots and water during the first twenty-five days of treatment, after which time a 1 per cent sodium chloride solution was substituted for the drinking water. Six days after this change the treated monkey—especially its neck and shoulder muscles—became very weak. It usually took up a characteristic posture, huddled up in a corner of the cage with the hands holding the back of the head. Within the next week, while the monkey was still taking saline solution, this condition became gradually worse, and finally paralysis was almost complete, so that the salt treatment had to be interrupted. Although by this time marked polyuria had developed, the monkey did not want to take water and hence had to be given milk. It recovered within two days. On the forty-third day of the experiment both the treated and the control animal received a total of 100 cc. of a 5 per cent sodium chloride solution in repeated intravenous injections within one hour. While the control tolerated this amount without any obvious indications of damage, the monkey treated with desoxycorticosterone acetate showed definite signs of weakness of the neck and took up the characteristic posture—with both hands holding the back of his head—immediately after the last injection of saline solution. Two hours later paralysis of a definitely spastic type, affecting practically the entire skeletal musculature, ensued. This condition was interrupted by epileptiform attacks of violent convulsions. Six hours later, however, the monkey recovered completely and took nourishment again. Two days after this a 1 per cent saline solution was given instead of drinking water. However, the animal showed a definite aversion to salt water and took little of it, attempting to get along on the water content of the carrots. Hence on the sixtieth day of treatment we removed the drinking water for one day to make the animal very thirsty, then gave a 3 per cent sodium chloride solution, and since this highly concentrated salt solution increased thirst even further, the monkey took large amounts of it. Within two days marked muscular weakness ensued, and on the third day deep coma developed, which resembled general anesthesia. In this condition motor responses to strong painful stimuli were not completely abolished but the corneal reflex disappeared. Although respiration was regular, the body temperature dropped progressively, and after a few hours the animal died. At autopsy the outstanding feature was again the enlarged white kidney, which weighed 17.2 Gm., while that of the control, killed at the same time, weighed only 12.3 Gm. The heart was also slightly enlarged (14.2 Gm., compared with 12.3 Gm. for the control), and the adrenal glands were somewhat subnormal in size, weighing 0.9 Gm., compared with 1.1 Gm. for the control. The other organs showed no significant changes. On histologic examination the most outstanding change was detected in the kidneys, in which the proximal and distal convoluted tubules were greatly enlarged. The height of the parietal lamina of Bowman's capsule was slightly increased. The most conspicuous change, however, was an invagination of the proximal convoluted tubules into the space of Bowman's capsules (fig. 3 *C* and *D*). Glomerular sclerosis was rarely observed, but most of the glomerular tufts were distinctly hyperemic.

Summarizing the salient features of this experiment one may say that a monkey weighing less than 3 Kg. was given a total of 2,520 mg. of desoxycorticosterone acetate over a period of two months. It showed clinically no conspicuous signs of damage except at times when it was simultaneously treated with high doses of sodium chloride. Under the influence of combined treatment with the salt and the steroid serious motor disturbances ensued, which finally resulted in death. Although the treatment caused polyuria, it did not result in marked retention of water such as is seen in fowl or in patients with Addison's disease receiving excessive doses of sodium chloride and desoxycorticosterone acetate. There were, however, marked signs of renal damage and compensatory adrenal atrophy.

Rat.—It is technically impossible to perform many experiments on large animals such as the dog or the monkey because of the enormous amount of costly desoxycorticosterone acetate required to produce symptoms of overdosage in them. Hence we decided to repeat these experiments in rats, and two series of investigations were performed in this species.

For our first experimental series we used three groups, each consisting of 6 rats. The average weight of these animals was 90 Gm. (range, 80 to 100 Gm.) at the beginning of the experiment. The animals in group 1 were not treated and served as controls, those in group 2 received 2 cc. of a 20 per cent sodium chloride solution once daily by stomach tube, while those in group 3 received in addition to this amount of saline solution 5 mg. of desoxycorticosterone acetate suspended in 0.2 cc. of water by subcutaneous injection twice daily. Water instead of oil was used as a carrier of the crystals because the comparatively large doses of oil which would have had to be injected in the case of these small animals are not well absorbed and tend to form subcutaneous "oil pouches." Treatment was continued at this same dose level for a period of twenty days. During this time no signs of damage, more particularly no evidence of edema, was detectable in any of the groups. It was striking, however, that while the muscular tonus in the salt-treated animals remained approximately normal, the group receiving desoxycorticosterone acetate plus saline solution showed signs of pronounced muscular weakness. Actual paralysis or anesthesia was not observed in this comparatively short experiment. However, the animals in the group receiving the combination treatment were soft and showed definitely low muscular tonus. At the end of the twenty days the rats treated with saline solution only, as well as those receiving saline solution plus desoxycorticosterone acetate, were placed in revolving cages having a diameter of 12 inches (30.5 cm.) and rotating at the speed of 16 revolutions per minute. After one hundred and five minutes of exercise only the animals treated with saline solution were still running vigorously, while most of the rats which had received saline solution plus desoxycorticosterone acetate exhibited signs of fatigue, 2 of them having completely collapsed. The latter 2 died within the next few hours after discontinuation of the forced exercise. At this time the other animals in all three groups were killed and the kidneys and heart in each instance weighed and histologically examined. The average weight of the kidneys of the controls was 1,241 mg., that of the group receiving saline solution only 1,334 mg. and that of the group with the combination treatment 1,443 mg. The average cardiac weights for the three groups were 668, 645 and 679 mg., respectively. Although the highest average renal and cardiac weights were in the group with the combination treatment, the differences were not of definite statistical significance. Histologically, however, hyperemia of the glomeruli and invagination of tubular cells into the cavity of Bowman's capsule were evident in most of the rats treated with desoxycorticosterone acetate and particularly prominent in the group receiving saline solution in addition to this steroid.

In the second experimental series we used two groups of rats weighing 120 Gm. on the average (range, 110 to 140 Gm.). One group remained untreated, while the other received 5 mg. of desoxycorticosterone acetate in 0.2 cc. of water subcutaneously twice daily. After six days of treatment all animals in both groups were given an intravenous infusion of a 5 per cent sodium chloride solution in a volume corresponding to 5 per cent of their body weight, in order to test the influence of pretreatment with desoxycorticosterone acetate on sensitivity to sodium chloride. None of the animals showed any serious sign of intoxication following injection of saline solution at this early stage of overdosage with desoxycorticosterone acetate. However, the average level of the blood chlorides (van Slyke's method) before the infusion of saline solution corresponded to 408 mg. of sodium chloride per hundred cubic centimeters in the treated, compared with 438 mg. in the control animals. This hypochloremia confirmed our previous observations. Six hours after the saline infusion second blood specimens were taken and found to contain a mean of 483 mg. per hundred cubic centimeters in the pretreated, compared with 448 mg. in the control group. The difference of the average rise in chloride concentration was found to be of high statistical significance when analyzed according to Fisher's [23] formula for small samples, P being less than 0.01. Following another week of daily treatment with desoxycorticosterone acetate, the same sodium chloride tolerance test was repeated on all animals after blood samples had been taken to determine the initial blood chloride concentration. By this time the treated animals became so sensitive to sodium chloride overdosage that 3 animals died within three to four hours after the injection. All rats pretreated with desoxycorticosterone acetate showed obvious motor disturbances after receiving the solution of sodium chloride, such as tremor, choreiform contractions and great muscular weakness. Six hours after the saline infusion the surviving animals were killed

23. Fisher, R. A.: Statistical Methods for Research Workers, ed. 5, Edinburgh, Oliver & Boyd, 1934.

together with the controls, and it was found that the blood chlorides had risen from the initial average level of 400 mg. to 517 mg. per hundred cubic centimeters in the controls and from 363 mg. to 626 mg. per hundred cubic centimeters in the pretreated rats. Here again statistical analysis indicates that the average rise in the treated animals was significantly higher that that in the controls ($P < 0.01$); that is to say that desoxycorticosterone acetate decreased the tolerance for sodium chloride. The brains of all the rats were collected at autopsy, and the average cerebral chloride content in the controls was found to be equivalent to 232 mg. of sodium chloride per hundred grams of fresh tissue and that in the group pretreated with desoxycorticosterone acetate was 275 mg. per hundred grams of fresh tissue. Here again the difference was statistically significant inasmuch as $P = 0.01$. It appears, therefore, that desoxycorticosterone acetate inhibits the elimination of excess sodium chloride and that at least part of this salt is retained in the brain. Further experiments will have to show whether this increase in electrolytes of the brain could be—at least in part—responsible for the motor symptoms of intoxication. The average weight of the kidneys of the controls was 1,221 mg. and that of the rats pretreated with desoxycorticosterone acetate was 1,339 mg. This apparent increase in renal size was not, however, of definite statistical significance. Histologically, glomerular hyperemia and invagination of cells of proximal convoluted tubules into the capsular space of the glomeruli were again prominent.

COMMENT

The most salient fact which emerges from all our experiments is that under suitable conditions desoxycorticosterone acetate can produce definite renal changes similar to those seen in human nephrosclerosis. These changes are most readily obtained in fowl, as shown by our earlier experiments, but they can also be produced in mammals. In the latter it is very probable that the refractory period is much longer than in birds and hence treatment has to be continued for a comparatively long time. In our experiments enormous doses of desoxycorticosterone acetate were given, but it is quite possible that similar and even more pronounced renal lesions may be obtained with much smaller daily doses if the treatment be continued over a longer period. It will be recalled that changes in the adrenal cortex have frequently been reported in association with arteriosclerotic and nephrosclerotic hypertension. Furthermore, adenoma of the adrenal cortex is often accompanied by a rise in blood pressure; hence it appears quite possible that chronic overdosage with endogenous cortical principles plays an important part in the genesis of nephrosclerosis in man.

On the other hand, our experiments also indicate that doses of desoxycorticosterone acetate, which are greatly in excess of those ever used in clinical medicine can readily be tolerated by the dog, the monkey and the rat as long as the intake of sodium chloride is kept within reasonable bounds. Simultaneous treatment with high doses of sodium chloride and desoxycorticosterone acetate results in potentiation of the symptoms of overdosage. Since the symptoms with the one agent are rather similar to those with the other, it is difficult to say whether one should speak of an accentuation of sodium chloride action by desoxycorticosterone acetate or vice versa. For this reason it appears preferable at the moment to speak merely of mutual synergism. This is certainly conspicuous with regard to the motor disturbances. In all three animal species examined during this investigation, motor disturbances could be elicited readily in animals pretreated with desoxycorticosterone acetate by administering sodium chloride in doses which proved innocuous for controls. Earlier investigations, discussed in the introductory section of this communication, revealed electrolyte changes in the muscles of animals overdosed with desoxycorticosterone acetate. The present experiments show, however, that at the time of the motor disturbances there also is marked derangement in the electrolytes of the brain. Further experiments will doubtless differentiate between the relative roles of the muscles and the nervous system in the production of motor

disturbances following overdosage with desoxycorticosterone acetate. It is tempting to assume a correlation between these motor disturbances and the general anesthesia which can be elicited by sudden flooding of the organism with this or other anesthetic steroids. It must be admitted, however, that up to the present time the nature of this correlation, if it exists, is still mysterious.

It is difficult to explain the great difference in the sensitivity of the various species to intoxication with desoxycorticosterone acetate and sodium chloride. It is noteworthy, however, that fowl, which are most sensitive to desoxycorticosterone acetate, are also much more sensitive to sodium chloride than mammals. It is conceivable that in birds the great sensitivity to sodium chloride is due to the fact that the reabsorption of glomerular filtrate is performed both by the tubules of the kidney and by the lining of the cloaca. Hence the power to concentrate urine is not as well developed in the avian as it is in the mammalian kidney. Perhaps, therefore, the renal tissue is especially sensitive to stimuli placing a great strain on its urine-concentrating ability. In any case, our findings concerning the decrease in chloride tolerance induced by desoxycorticosterone acetate are in agreement with similar observations made by Piantoni and Orias [24] in the rabbit and may help to explain the increased effectiveness of sodium chloride in the organism pretreated with desoxycorticosterone acetate.

The adrenal involution caused by high doses of desoxycorticosterone acetate in the various species is merely another example of compensatory atrophy induced in a hormone-producing gland by overdosage with a principle normally made by its cells. It is difficult to explain the participation of the medulla in this compensatory atrophy, since in our experiments the overdosage was produced with a compound having the action of a cortical hormone. It has often been claimed, however, that the medulla participates in the elaboration of cortical hormones and that the peculiar vascular arrangement of the gland is specially designed for this purpose. Most of the arterial blood reaches the cortical cells first, and the medulla is almost exclusively supplied by venous blood coming from the cortical sinusoids. It is not inconceivable that such a vascular arrangement has been established in order to supply the medulla with hormonal precursors coming from the cortex. In any case atrophy of the cortex is likely to affect the medulla because of the peculiar blood supply of the latter.

In conclusion it should be emphasized that none of the changes which we consider to be characteristic of overdosage with desoxycorticosterone acetate could have been caused by the sodium chloride given to our experimental animals since the controls always received the same amount of this salt. It is possible, however, that some or all of the morphologic changes were accentuated by the simultaneous treatment with sodium chloride. This was clearly demonstrable in the motor disturbances, which could always be elicited by sodium chloride in animals receiving a constant daily supply of desoxycorticosterone acetate, while they disappeared after withdrawal of sodium chloride, even though treatment with the steroid was continued.

SUMMARY

The characteristic morphologic and functional changes caused by overdosage with desoxycorticosterone acetate, a synthetic preparation having the properties of a hormone of the adrenal cortex, have been studied in the dog, the monkey and the rat. It has been found that in none of these species does any marked retention of water or edema of tissue develop even if enormous doses are given (40 mg.

24. Piantoni, C., and Orias, O.: Rev. Soc. argent. de biol. **18**:326, 1942.

per day for small dogs or monkeys and 10 mg. per day for rats) over a period of several weeks or months. Even simultaneous treatment with large doses of sodium chloride failed to cause significant retention of water in any of these species. On the other hand, severe motor disturbances, which may progress to complete paralysis and death, result in all these species if sodium chloride is administered following pretreatment with such high doses of desoxycorticosterone acetate. Withdrawal of sodium chloride causes the motor disturbances to disappear in spite of continued administration of desoxycorticosterone acetate. The possibility of a correlation between the motor disturbances and the anesthetic effect of this and other steroids has been emphasized.

The most striking morphologic changes were observed in the kidneys. In the monkey and the rat there is a curious invagination of cells of the proximal convoluted tubules into the space between the visceral and the parietal layer of Bowman's capsule. This change has not been observed in the dog; however, in the latter sclerosis of individual glomeruli and of the connective tissue immediately surrounding them was more pronounced than in the other species. Hypertrophy of the proximal and the distal convoluted tubules and obliteration of some tubular lumens by desquamated epithelial cells or hyaline casts were noticeable in all three species. It was striking, however, that mammals are particularly resistant to nephrosclerosis and generalized edema in comparison with fowl which, in turn, are more resistant to the motor disturbances caused by overdosage with desoxycorticosterone acetate.

The morphologic changes which are induced in other organs by excessive doses of desoxycorticosterone acetate have also been described.

Following the administration of sodium chloride to rats pretreated with desoxycorticosterone acetate there is a more significant rise in the concentration of chlorides in the brain and blood than in animals not pretreated but given the same amount of sodium chloride. Apparently this steroid increases retention of chlorides.

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NATURE OF THE HYALINE CHANGES IN ISLANDS OF LANGERHANS IN DIABETES MELLITUS

J. B. AREY, M.D.
MINNEAPOLIS

Since Opie's original description of hyaline degeneration of the islands of Langerhans [1] various authors have expressed different views as to the source of the hyaline material. Three interpretations seem possible: (*a*) It may have its origin in primary degeneration of the epithelial cells of the islands; (*b*) it may result from hyaline degeneration of pericapillary connective tissue; (*c*) it may represent deposition of amyloid. The purpose of this study was to determine the source and the nature of this hyaline material and to decide whether the degeneration of the insular cells is a primary or a secondary process.

Is the hyaline material of epithelial origin? Opie [2] and Wright and Joslin [3] described the hyaline material as originating from primary degeneration of the epithelial cells. Although at first Opie [2] was inclined to believe that it was formed by a process resembling secretion, he later decided that it was of epithelial origin.

Warren [4] stated the following arguments against an epithelial origin for the hyaline material: (*a*) If this substance occurs as a result of degeneration of the island cells, it should occur as frequently in young as in older diabetic patients, yet 96.6 per cent of his 89 patients were persons over 40 years of age; Kraus [5] and Weichselbaum [6] also pointed out a definite relationship to the older age groups. (*b*) The blue-staining island cells described by Opie [2] are said to occur in non-diabetic persons and in those organs that are without hyaline islands. They do not show the definite relationship to the blood vessels that true hyalin does. Bloom [7] described blue-staining cells as normal constituents of the islands, designating them as D cells. (*c*) Amyloid reactions have been obtained in a number of cases (14 of 51) with hyaline islands. (*d*) Serial sections show that the hyaline material is always in contact with the walls of vessels and is always intercellular. (*e*) If the hyaline material is the result of cell degeneration secondary to diabetes, it should not occur in the absence of the latter. Warren concluded that the hyaline material was the result of the production of intercellular substance by fibroblasts and possibly by endothelial cells. When sufficient epithelial cells were destroyed or separated from the blood stream, diabetes resulted.

Is the hyaline material formed from pericapillary connective tissue? The majority of investigators, including Weichselbaum and Stangl,[8] Herxheimer,[9]

From the Department of Pathology, University of Minnesota.

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8. Weichselbaum, A., and Stangl, E.: Wien. klin. Wchnschr. **14**:968, 1901.
9. Herxheimer, G.: Virchows Arch. f. path. Anat. **183**:228, 1906.

Kraus,[5] Schmidt,[10] Gibb and Logan,[11] Mallory [12] and Warren,[4] have not believed that the hyaline material is of epithelial origin. Opie [13] located the structureless hyaline masses between the capillary vessels and the parenchymal cells, which formed narrow compressed rows between adjacent columns of hyalin. Weichselbaum and Stangl [8] in 1901, without knowledge of Opie's work, described thickening and homogeneity of the island stroma resembling an obliterated renal glomerulus. Later Weichselbaum [6] showed that this could be distinguished from the connective tissue proliferation extending from the surface of the island. He attributed the hyaline degeneration to swelling of the pericapillary connective tissue and formation of a homogeneous material in the latter, with resultant atrophy of the epithelium. The end stage was a non-nucleated avascular structure. Herxheimer [9] expressed the belief that the earliest hyaline change was the appearance of a slightly thickened nucleated connective tissue within the islands. Gibb and Logan [11] stated that advanced fibrosis was always present with hyalinization and must be considered as a precursor of hyaline degeneration. Gellé [14] expressed the belief that the hyaline material was formed by degeneration of both connective tissue and epithelium. According to Mallory,[12] it originates in the same manner as amyloid, namely, as a secretion of the fibroblasts outside the walls of the island capillaries, with resultant atrophy and disappearance of the islet cells by pressure.

Is the hyaline material of amyloid nature? Mallory stated that the hyaline material in the islands of Langerhans sometimes gave the characteristic amyloid staining reaction with methyl violet and at other times did not. He stated that it probably was closely related to, even if not identical with, amyloid.

Warren obtained amyloid reactions with methyl violet or iodine green in 14 of 51 cases of hyalinized islands.

Gellerstedt [15] stated that insular amyloidosis was identical with so-called hyaline degeneration, at least as far as staining reactions were concerned. The pancreases from 181 consecutive postmortem examinations were used as a source of material, representing 110 persons between the ages of 50 and 90 years. They were stained with methyl violet, congo red and Van Gieson's mixture. The microscopic appearance was identical with that of hyaline degeneration, the deposits being pericapillary, located between the membrana propria and the endothelial cells of the capillaries. Occasional ring deposits of amyloid occurred, and the epithelial cells were strongly compressed. The distinction from general amyloidosis was readily made, as in the latter many vessels other than the insular capillaries are involved. In 46.3 per cent of the cases staining for amyloid within the islands gave positive results, but the large majority of sections showed little or almost no amyloid. The amount of insular amyloidosis did not appear to follow the intensity of generalized atherosclerosis, and no causal relationship could be established between the amyloid and pancreatic arteriosclerosis or arteriolosclerosis. Although these patients were not rigidly studied clinically for the possible presence of diabetes, only 3 were known to have been diabetic. The author expressed the belief that the pancreatic localization of the hyaline material was only a form of senile deposition of amyloid such as is said to occur in the seminal vesicles and in the brain in senile psychosis (Alzheimer's disease).

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10. Schmidt, M. B.: München. med. Wchnschr. **49**:51, 1902.
11. Gibb, W. F., Jr., and Logan, V. W.: Arch. Int. Med. **43**:376, 1929.
12. Mallory, F. B.: The Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1914, p. 521.
13. Opie (footnotes 1 and 2).
14. Gellé, cited by Kraus.[5]
15. Gellerstedt, N.: Beitr. z. path. Anat. u. z. allg. Path. **101**:1, 1938.

Van Beek [16] investigated 59 cases of diabetes mellitus. The patients ranged in age from 5 to 85 years. Of these, 40 per cent showed amyloid deposits in the islands of Langerhans. The deposits were found only in persons over 50 years of age, the percentage increasing with age. He expressed the belief that the hyaline material was actually an amyloid deposit, not a part of generalized amyloidosis but a local deposit. He found amyloid in the pancreases of some aged persons without diabetes. He expressed the belief that he could demonstrate parallelism with arteriosclerosis and especially with arteriolosclerosis but was unable to determine whether the deposits were the cause or the result of the diabetes. He stated that the factors leading to the precipitation of the amyloid were still unknown.

Rubarth [17] described amyloid in the islands of Langerhans of a 10 year old angora cat with glycosuria.

MATERIAL AND METHODS

Source of Material.—Two hundred pancreases from persons 72 hours to 87 years old were examined. Fifty-two of these subjects were known to have been diabetic, no positive diabetic history was recorded for 148. Eighty-four were females, and 116 were males. Only 40 persons were under 50 years old.

Stains and Fixatives.—As fixatives, 4 per cent solution of formaldehyde, alcohol, Bensley's, Zenker's and Helly's solution were tried. The best results were obtained with Helly's or Zenker's solution and solution of formaldehyde, and these alone were finally employed. As stains, Bensley's neutral gentian violet, hematoxylin and eosin, iron-hematoxylin counterstained with Van Gieson's stain and the Mallory-Heidenhain stain were employed. The latter was by far the most satisfactory for demonstrating hyalin and its relations and was used exclusively in the later sections. Forty-two sections were stained with methyl violet in an attempt to demonstrate amyloid. Only those sections showing sufficient hyalin to allow recognition with this stain were thus treated. Congo red and gentian violet were also used, but these stains failed to give as exact color distinctions as methyl violet.

OBSERVATIONS

First Appearance of Hyaline Substance.—The hyaline material first appears as rounded, smooth globular or elongated masses immediately outside the capillary basement membrane (*A* in figure). These masses take a distinct pale blue color with the Mallory-Heidenhain stain and occasionally show fine radial striations. Hyaline masses are sometimes seen which appear to follow the course of the capillary basement membrane but are separated from it by a small clear space. This space is probably the result of shrinkage, since the hyaline material when followed in serial sections always makes contact with the basement membrane. Isolated hyaline masses apparently bearing no relation to the capillaries are not infrequent, but in serial sections they are found to represent portions of pericapillary deposits. In a number of pancreases both with and without hyaline islands blue-staining cells like those described by Opie could be seen. However, in no instance could transitions between these and true hyaline deposits be found.

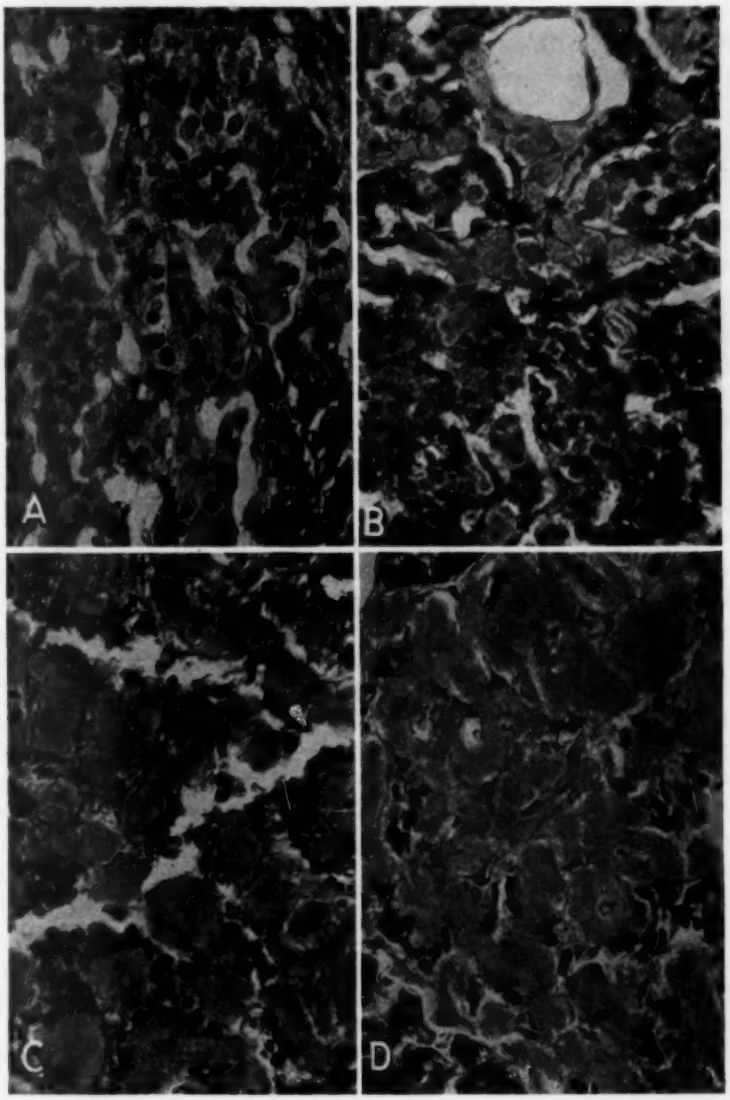
Increase in the Amount of Hyaline Material.—The hyaline material increases in amount either by the formation of new globular masses or by an increase in size of the preexistent masses. Frequently a larger mass appears to be composed of fused globules which are still separated by delicate lines (*B* in figure).

The Advanced Stage.—As the hyaline material increases, the epithelial cells become more compressed and distorted, their cytoplasm appearing scantier and their nuclei more pyknotic (*C* and *D* in figure). In the advanced stage definite distinc-

16. Van Beek, C.: Nederl. tijdschr. v. geneesk. **83**:646, 1939.

17. Rubarth, S., cited by Gellerstedt.[15]

tions can no longer be made between epithelial, endothelial and connective tissue nuclei. The atrophy may involve only a part of an island. In the end stage of hyalinization the picture is not unlike that of an advanced stage of amyloid disease of the liver. Cords of hyaline substance, having the form of the original epithelial cords of the islands, are separated by capillaries.



A, photomicrograph of a few small amyloid deposits in an islet of Langerhans of a diabetic man 76 years old. The pericapillary position of the amyloid is shown in the deposit at the center of the right margin.

B, photograph from the same case as *A*, showing moderate amyloidosis with pericapillary distribution.

C, photomicrograph of an extensive amyloid deposit in an island of Langerhans, with atrophy of the epithelial cells of the island, in a diabetic man 64 years old. There is a striking resemblance to amyloidosis of the liver.

D, photomicrograph from the same case as *C*, showing extreme amyloidosis of an islet of Langerhans.

Disappearance of the Capillary Basement Membrane.—The disappearance of the capillary basement membrane does not depend entirely on the amount of the hyaline deposit, since a small hyaline accumulation sometimes blends with the basement membrane, but in general the basement membranes are more readily seen with small than with large hyaline deposits. The disappearance of the capillary basement membrane may be due to blending with the amyloid, similar to that described by Bell [18] in the glomeruli of the kidney in amyloid disease.

In only one of the sections examined were calcium deposits present such as those described by Opie [2] and others.[11]

Amyloid Staining Reactions.—Of the 55 pancreases in the present series showing hyaline islands, 42 had fair amounts of hyaline substance and were stained with methyl violet. All of these 42 gave positive reactions for amyloid. The hyaline deposits gave a distinct purple reaction with methyl violet, such as is seen in amyloid deposits in the liver, spleen, kidney and adrenal glands in cases of generalized amyloidosis.

General Features of Amyloidosis.—In cases of generalized amyloidosis the deposits in the spleen are located between the sinusoids, in the pulp spaces or in the malpighian corpuscles. In the liver and the adrenal glands they are located just outside the sinusoidal endothelium. In the liver there is gradual atrophy of the hepatic cords until these may be completely replaced in some areas by amyloid. With extensive involvement there is still retention of the sinusoidal pattern. The deposits of amyloid in the glomeruli of the kidney differ somewhat from the hyaline deposits in the islands of Langerhans. According to Bell,[19] the deposits in the former are located within the capillary basement membrane, between it and the scattered endothelial cells. Thus although the relationship to capillary endothelium of the hyaline masses of the islands is similar to that of glomerular amyloid deposits the relationship to the capillary basement membrane is altered. The hyaline deposits in the pancreas appear to resemble more closely amyloid deposits in the liver, spleen and adrenal glands than those in the glomeruli of the kidney.

Neither the source nor the nature of amyloid is definitely known. Since the chief inciting cause of amyloidosis is chronic suppuration, it has been suggested that amyloid is a product of protein cleavage. Leupold, Kuczynski and others, according to Métraux,[20] stated that amyloid is the gel form of a protein, related to a globulin. Bell expressed the belief that amyloids from different sources are not identical in chemical nature; accordingly, until more exact microchemical analyses can be used, proof of the amyloid nature of any given substance must depend on staining reaction and manner of deposition.

Comparison of Insular Hyaline Deposits and Known Deposits of Amyloid.—In amyloid disease of the liver the amyloid deposit stains a deeper color with methyl violet than does the hyaline deposit in the islands of Langerhans. With hematoxylin and eosin amyloid deposits in the liver were a brighter pink than were hyaline deposits in the pancreas. Moreover, the latter did not have so glassy and homogeneous an appearance as amyloid in the liver. However, if several sections of liver were examined, variations were also noted here in depth of staining reaction, apparently dependent on the amount of the deposit and its composition. With the Mallory-Heidenhain stain the same variations were noted, the hyaline material in the pancreas being a lighter blue and often having a faintly striated or stippled appearance.

18. Bell, E. T.: Am. J. Path. **9**:185, 1933.
19. Bell, E. T.: Textbook of Pathology, ed. 4, Philadelphia, Lea & Febiger, 1941, p. 108.
20. Métraux, P.: Frankfurt. Ztschr. f. Path. **37**:279, 1929.

A small number of cases of known generalized amyloidosis were examined in order to determine whether amyloid deposits occurred in the islands of Langerhans, and, if so, how they compared with the "hyaline degeneration" of the islands in diabetes. In a number of sections amyloid deposits were found in the islands, the acinous tissue and the walls of vessels which when stained with hematoxylin-eosin or methyl violet resembled the deposits in the liver and the spleen. The insular amyloid in generalized amyloidosis is a little more glassy in appearance than the hyaline material of diabetes, and it stains somewhat deeper with methyl violet, but the resemblance is close. It cannot be said that the insular hyalin in diabetes and that in general amyloidosis are identical chemically, but they are similar in staining reaction and in manner of deposition around the capillaries.

"Hyaline Degeneration" in Nondiabetic Persons.—Until 1904 hyaline degeneration was considered a specific change of diabetes mellitus. In that year both Ohlmacher [21] and Sauerbeck [22] described hyaline islands in nondiabetic persons. Similar findings were reported by Karakascheff [23] in 1905 and by Saltykow [24] in 1909. Cecil [25] in 1914 reported 6 cases of hyaline degeneration of the islands occurring in nondiabetic patients, all over 37 years old. He stated that the hyalin appeared to originate about thickened insular capillaries, and he expressed the belief that the change might possibly be referable to sclerosis of the smaller vessels. Wright [26] reported 5 nondiabetic patients showing hyaline islands. All were over 50 years of age and were considered prediabetic. Warren [27] gave the incidence of hyaline islands in 200 nondiabetic pancreases as 2 per cent. Gellerstedt's and Van Beek's work has been mentioned.

In the present series no qualitative difference was noted between the hyalin of persons known to be diabetic and that of persons without diabetes. Of the 114 nondiabetic patients over 50 years old, 19 (16.6 per cent) showed some hyaline deposits in the islands. Often only one island in a section showed partial hyalinization. Of the 34 nondiabetic patients under 50 years of age, none showed hyaline islands. Of the 46 patients with diabetes who were over 50 years old, 33 (71.7 per cent) showed hyaline islands, compared with 3 (50 per cent) of the 6 with diabetes who were under 50 years of age. The involvement of the islands was usually much more extensive in the diabetic than in the nondiabetic persons. It must be remembered, however, that diabetic histories might be lacking in a few cases.

COMMENT

The exact relationship between hyalinization of the islands of Langerhans and diabetes mellitus has not been determined. It seems apparent that the hyaline deposits are not the result of diabetes per se, but when extensive they may cause or accentuate diabetes. There is, however, no constant relation between the clinical severity of the diabetes and the extent of hyalinization. Warren [4] stated that diabetes associated with hyaline islands is apt to be mild. The possibility that the deposits are entirely independent of diabetes must also be considered. It has been previously mentioned that they are far more frequent in the older age groups. The one factor that diabetes and old age have in common is vascular disease. This is known to be far more severe in diabetic than in nondiabetic patients of the same

21. Ohlmacher, J. C.: Am. J. M. Sc. **128**:287, 1904.
22. Sauerbeck, E.: Ergebn. d. allg. Path. u. path. Anat. **8**:654, 1904.
23. Karakascheff, K. I.: Deutsches Arch. f. klin. Med. **82**:60, 1905.
24. Saltykow: Cor.-Bl. f. schweiz. Aerzte **39**:625, 1909.
25. Cecil, R. L.: Am. J. M. Sc. **147**:726, 1914.
26. Wright, A. W.: Am. J. Path. **3**:461, 1927.
27. Warren, S.: The Pathology of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1930, p. 71.

age. Therefore, it is possible that hyalinization of the islands is not dependent on diabetes but is merely a senile change depending on vascular disease and therefore accentuated by one of the complications of diabetes. The fact that the hyaline deposits are less frequent in younger diabetic persons favors this view.

SUMMARY

"Hyaline degeneration" of the islands of Langerhans is actually local deposition of amyloid. The amyloid is pericapillary in distribution, never intraepithelial. Amyloid deposits in the islands of Langerhans are far more frequent in the older age groups.

There is no qualitative difference between the amyloid deposits in persons with and persons without diabetes. Amyloid deposits in the islands of Langerhans occur in 16.6 per cent of nondiabetic patients and 71.7 per cent of diabetic patients over 50 years of age.

The amyloid in the islands of Langerhans may not be identical with the amyloid in the liver and the spleen in cases of generalized amyloidosis.

PORTAL-SYSTEMIC COLLATERAL VEINS IN THE GUINEA PIG WITH SCHISTOSOMAL CIRRHOSIS OF THE LIVER

AND A DISCUSSION OF CONGESTIVE SPLENOMEGALY

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The character and the extent of portal-systemic venous anastomoses arising on the basis of portal hypertension are well known in human pathology. Little attention, however, has been paid to their distribution and comparative significance in animals in which such hypertension has been directly or indirectly produced.

In studies on experimental schistosomiasis in rats we [1] were struck by the large numbers of adult worms which migrated from the portal venous system, their natural habitat, to the right side of the heart and to the lungs. In later experiments with guinea pigs, in which a similar migration occurred, we had the opportunity of studying the development of the portal-systemic collateral veins, as well as their regression in the course of the evolution of the disease, while worms could be seen migrating through them.

A detailed description of portal-systemic venous anastomoses in small laboratory animals with experimental cirrhosis of the liver has not been encountered in the available literature. Our knowledge of some of these anastomoses has been largely derived from studies in normal animals that had been submitted to various experimental procedures, such as ligating and injecting the portal vein under maximum pressure (Gilbert and Villaret [2]), repeating the same procedure and tying the venae cavae and the aorta (cited by Weiss [3]) or ligating the portal vein and/or the inferior vena cava in the living animal and observing the anastomoses at a later date (Gilbert and Villaret [2]; Neuhof [4]; Rous and Larimore [5]; Drury [6]; McMichael and Smirk [7]; Stephenson [8]; Boyce, Lampert and McFetridge [9]). The latter procedure, however, introduces the factor of postoperative adhesions and the formation of new vascular pathways tending to minimize the collateral channels that would ordinarily have been established. It seemed worth while to describe these anastomoses as they appear in the intact animal with

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† W. A. Hoffman died on April 4, 1943.

1. Krakower, C.; Hoffman, W. A., and Axtmayer, J. H.: Puerto Rico J. Pub. Health & Trop. Med. **16**:269, 1940.
2. Gilbert, A., and Villaret, M.: Arch. de méd. expér. et d'anat. path. **18**:422, 1906.
3. Weiss, S.: Internat. Clin. **1**:148, 1936.
4. Neuhof, H.: Surg., Gynec. & Obst. **16**:481, 1913.
5. Rous, P., and Larimore, L. D.: J. Exper. Med. **31**:609, 1920; **32**:249, 1920.
6. Drury, D. R.: J. Exper. Med. **49**:759, 1929.
7. McMichael, J., and Smirk, F. H.: J. Path. & Bact. **37**:81, 1933.
8. Stephenson, G. W.: Arch. Path. **14**:484, 1932.
9. Boyce, F. F.; Lampert, R., and McFetridge, E. M.: J. Lab. & Clin. Med. **20**:935, 1935.

marked intrahepatic portal obstruction, to stress their importance in schistosomal infections and to correlate the observations with those in congestive splenomegaly.

An account of the life history of the schistosomes can be readily obtained in any of the standard textbooks on helminthology. Suffice it to say that in the case of Schistosoma mansoni, the type with which we are concerned, the cercarias shed by infected snails (australorbis) penetrate the skin of the host, enter the blood stream and by way of the lungs reach the hepatic portal system, where they mature and, between the fifth and sixth weeks after infection, begin to deposit ova. The adult worms migrate into the subserosal and submucous venous plexuses of the small and the large intestine, where they deposit their ova in the different coats of the intestinal wall. In man and monkeys ova are liberated into the intestinal lumen but in lower animals few if any ova are found in the stools. While such oviposition occurs within the liver (at least in lower forms) by migration of the parasites within intrahepatic radicles of the portal vein, many of the ova encountered in the liver are embolic, having drifted with the blood stream from foci of oviposition elsewhere. In man, in addition to dysenteric symptoms, hepatic cirrhosis with congestive splenomegaly often results, giving rise to a clinical picture similar to that of Banti's disease.

The procedure followed in infecting guinea pigs was similar to that previously described with respect to rats. Young guinea pigs weighing between 200 and 400 Gm. were each exposed to between 3,500 and 4,700 cercarias, the numbers varying with different lots. One hundred and thirty-nine animals were available for observation of the portal-systemic collateral veins, but attention here will be confined to the pertinent gross and histologic morphologic changes underlying the portal hypertension in this parasitic disease in those infected control animals which were fed on grass; the findings in the animals kept on a deficient diet will not be dealt with here.

GROSS OBSERVATIONS

In the early stages of infection there was an increase in the weight of the liver without a proportionate increase in the weight of the body. During the height of infection, when portal-systemic collateral veins were well established, the ratio of the weight of the liver to that of the body was not appreciably altered, but both weights were generally reduced. In the late stages, after three hundred days, the weights of the body and the liver were about double the original weights. Before oviposition the liver revealed some opaque yellow lesions. After oviposition the following changes were noted: Capsular pitting and white or yellow flecks or streaks alone were common between the forty-ninth and ninety-second days. On the one hundred and fifth day a diffuse nodular type of cirrhosis was superimposed. This cirrhosis was no longer apparent after three hundred days. Either total lobar (chiefly left and left accessory) or marginal atrophic areas (all lobes), rarely on the basis of earlier verminous thrombosis of the extrahepatic divisions of the portal vein, were in evidence from two hundred and thirty days onward and persisted as late as nineteen months after infection, when the last of the animals was killed. In these late stages few scars or flecks were seen. Grayish discoloration of the liver, due to the deposition of parasitic pigment (representing the regurgitated contents from the guts of the parasites), became intensified with the earlier progress of the infection, but practically cleared in the late stages. Ascites was never found in guinea pigs on the normal diet.

The weight of the spleen generally was slightly above, not infrequently double and on a few occasions triple the normal weight. Except for gross parasitic pigmentation of the spleen, there were no other notable features.

MICROSCOPIC OBSERVATIONS

The microscopic changes in the liver were essentially those of endophlebitis and periphlebitis involving the larger thick-walled muscular, as well as the perpiheral thin-walled nonmuscular, intrahepatic radicles of the portal vein.

Associated with the widespread intrahepatic migrations of the parasites prior to oviposition, subendothelial and perivascular lymphocytic and eosinophilic infiltration of the smaller radicles was observed. Such intensified infiltration combined with focal intravascular histiocytic prolif-

eration often marked the destruction of some of these worms, which at this inmature stage were readily digested.

The reaction provoked by the schistosomal ova consisted of (1) an intense eosinophilic and lymphocytic response, particularly about recently deposited ones with viable embryos, (2) histiocytic and fibroblastic nodular proliferation with giant cells about the ovum proper, most pronounced when associated with organization of parenchymatous necroses but also as a later stage of the eosinophilic and lymphocytic response, and (3) simple giant cell reaction about and often within the ovum, most often seen about older ova. Various combinations and degrees of these three types of reaction were often encountered at the same time. In all sites, except when the ova could escape into the lumen of the gastrointestinal tract, the inner embryonic mass might undergo a certain amount of development but was invariably destroyed. Older ova enclosed within giant cells became folded and wrinkled much as a piece of cellophane wrapping squeezed in one's hand.

The adult parasites generally wandered within the larger intrahepatic portal radicles, owing to their increased size. The thick-walled muscular vessels and their immediate wide thinner-walled branches responded to these migrations by intimal cellular infiltration and mural thrombosis, resulting eventually in intimal histiocytic and fibroblastic proliferation and thickening. Destruction of parasites called forth either a necrotizing thrombotic inflammatory reaction with massive eosinophilic infiltration, followed by histiocytic, giant cell and fibroblastic organization or slower organization of the hyalinized necrotic sector of worm by the latter type of response. Often both types of reaction were present in different sectors of the same vessel and about the same dead worms. In these larger vessels ova were more frequently trapped where endovascular lesions were well advanced and the lumen was appreciably narrowed or canalized.

As a result of these several processes, profound changes occurred within portal spaces and parenchyma after the onset of oviposition.

In the peripheral portal spaces, where the embolic ova lodged in largest numbers, progressive thickening occurred, at first locally and then more generally, reaching a peak of reaction about one hundred and forty-eight days after infection. By this time the spaces were heavily infiltrated either by lymphocytes (with occasional formation of genuine lymphoid follicles, including secondary centers and sometimes ova) or by eosinophils or by both, the infiltration presenting irregular cellular extensions into the adjacent parenchyma (fig. 1*A*). The ova within these spaces were irregularly massed and were surrounded most frequently by either histiocytic nodules or simply by giant cells. The venous radicles were obliterated in areas and generally irregularly narrowed, although there were wide patent sectors. There was increased vascularity throughout the portal space, characterized by prominent capillaries, wide juxtasinusoidal venous tributaries or enclosed widened hepatic sinusoids themselves. Bile capillaries proliferated freely, particularly about the peripheries of these spaces, extending irregularly into the lobules. A dense reticular mesh and collagenous scars were accompanying features. With progressive destruction of the parasites within the liver and to a lesser degree in other sites (e. g., within the parietes of the intestine) and with migration of the worms in large numbers through established portal-systemic collateral veins, fewer and fewer ova reached the liver in later stages of the infection. In consequence, resolution of the lesions and in great part restitution to integrity in these peripheral portal spaces occurred earlier and were generally more complete than in the larger more central ones. Infiltrating cells largely or totally disappeared. Since ova practically never became calcified, they were absorbed. At no time, however, was it possible to trace all the stages leading to their disappearance except for progressive shrinkage in size, distortion and thinning of their shells. The venous radicles were now patent again, although here and there a fibrous or more cellular endovascular septum or shelf persisted. The dense reticular and fibrous or hyaline scars were largely or totally absorbed.

The larger portal spaces likewise revealed partial or complete occlusive changes of their thick-walled muscular veins of maximum intensity about one hundred and sixty-three days after infection. At any one stage, however, progressive and regressive processes were apparent at the same time, either largely intimal or, when parasitic destruction and thrombosis occurred, intravascular. The intravascular reaction either was a recent formation of granulation tissue, with heavy lymphocytic and eosinophilic infiltration, or was more predominantly histiocytic and fibroblastic. Canalization was either slight or marked. The enlarged, distended vessels of the earlier reactive stages became shrunken and contracted in the later healing stages. As a result, the veins either were completely obliterated, being occupied by more cellular or more hyalinized connective tissue, or were partially patent and elsewhere threaded by small or larger endothelium-lined channels, often enclosing a hyalinized nodule, the organized remains of necrotic worms or ova. The medial coats were often spared, although frequently involved to a varied degree. The adventitial tissues were generally much less involved here than in the peripheral

portal spaces although showing, too, varied degrees of cellular infiltration and fibrous tissue increase. In the very late stages of the infection considerable resolution occurred in these portal spaces as well, particularly in the diaphragmatic and central portions of the liver. A common persistent feature was either uniform or eccentric intimal fibrous thickening of the veins (fig. 1 *B*). In submarginal and marginal areas associated with parenchymatous atrophy some active lesions were still present, while totally fibrosed and obliterated veins and partially fibrosed and canalized ones with spurs and septums were not uncommon. By contrast, except for the juxtahilar divisions of the portal vein, i. e., its lobar branches, which might be involved, the portal, superior mesenteric and generally inferior mesenteric veins showed no appreciable microscopic changes, though they were often stuffed with worms in earlier stages of the infection.

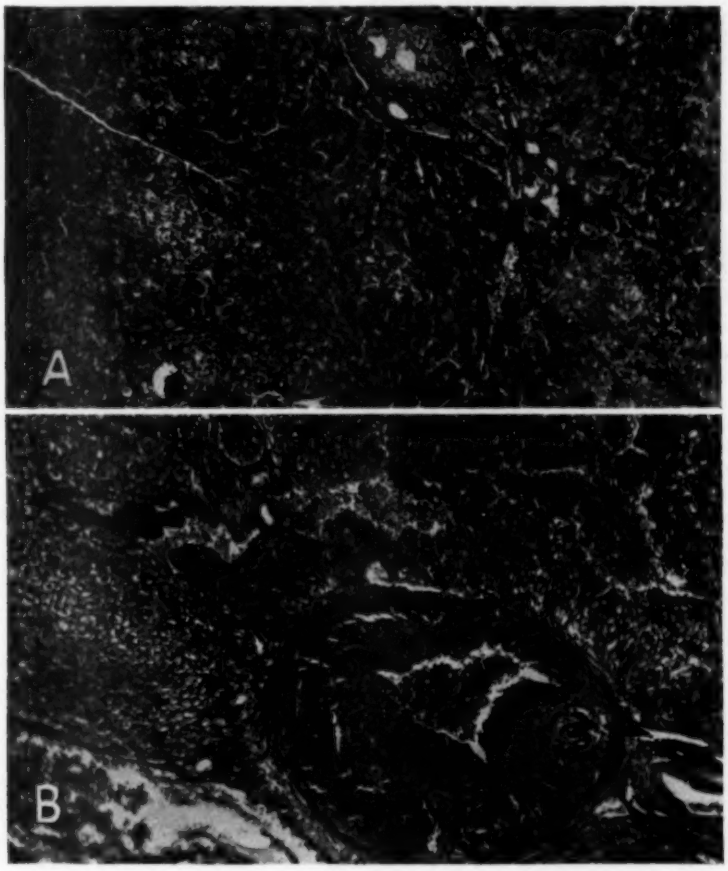

Fig. 1.—*A*, reaction about ova in peripheral portal spaces, with obliteration of the venules in these spaces. The lighter areas within the parenchyma represent irregular foci of hyperplasia. × 80. *B*, thick-walled muscular intrahepatic portal vein with marked proliferative endophlebitis. Above and to the left of it may be seen a branch with septum and shelf formation. × 80.

As for the hepatic parenchyma, necroses were a constant feature throughout all stages. They were often extensive and periportal, associated either with ovular embolism in peripheral venules or with sectors of veins the seat of reaction to destroy parasites. In the former instance, best seen about the seventy-eighth day after infection, the hyaline necrotic areas underwent organization by the ingrowth of hypertrophied histiocytes and the irregular proliferation of bile capillaries. Ova were often found within such necrotic and organizing areas. These organized lesions became incorporated within the widened portal space, the more so as they regressed and atrophied, leaving a thick reticular, fibrous or hyaline nodular scar within the confines of the portal space proper. These scars were finally absorbed. The

necrotic areas associated with parasitic destruction were often heavily infiltrated by polymorphonuclears, eosinophils and lymphocytes and were either completely replaced by regenerated liver cells or, infrequently, organized by fibrous tissue. Otherwise necroses occurred irregularly throughout the lobules. They were at times rapidly handled by autolysis, phagocytic removal of necrotic debris and regeneration from surrounding liver cells. Otherwise, when hyaline, with delay or absence of autolysis, they were slowly organized as already described, except that giant cells might be formed about the necrotic liver cells and proliferation of bile capillaries might be absent. In such instances regeneration of liver cells was tardy. Either regenerating liver cells filled in at the periphery of the contracting organized lesion or permeated through it. In consequence of these necrotic changes, with retarded regeneration, and, apparently, the profound vascular disturbances leading to alterations in the pattern and the rate of blood flow (due to interlobular portal venous obstructive lesions, including their juxtasinusoidal radicles), intralobular nodular hyperplasia of liver cells occurred. There was, however, little distortion of the portal-central vein relationship. These hyperplastic nodules were often irregularly coursed by bile capillaries. They were first recognized ninety-two days after infection and disappeared after the three hundredth day, when a normal arrangement of liver cell cords was reestablished.

Aside from these changes there were at all stages irregular extensions of bile ducts from the portal spaces into the adjacent parenchyma, occasionally accompanied by dilated sinusoids and some connective tissue, resembling in all newly formed portal spaces. There were also, particularly in the later stages, intralobular, often pericentral areas of pseudoadenomatous proliferation of bile ducts, with reticular or collagenous fibrosis and some deposition of parasitic pigment. These lesions appeared to resolve slowly, as the bile ducts connecting with adjacent liver cells seemed to be functional components, for a time at any rate. Whether in all instances these ducts communicated with bile capillaries in portal spaces was not determined. Lodgment of ova within widened hepatic sinusoids was sufficiently frequent to make one believe that some of these intralobular residuals, such as pseudoadenomatous biliary scars and healing necroses, were the result of earlier necrotic and reactive changes due to their presence there.

The atrophic subcapsular or marginal areas of the liver often revealed marked fatty parenchymatous changes with collapsed fibrosed portal spaces, enclosing tortuous bile ducts, which were occasionally distended by inspissated bile. There were also irregular intralobular scars.

The hepatic veins were little involved but in late stages often did present considerable adventitial fibrosis.

Parasitic pigment, which was taken up by Kupffer cells and by the histiocytes of portal spaces and scars, gradually disappeared in later stages of the infection, having been either transported to the biliary lymph nodes, the lungs, the spleen or other sites or chemically altered in situ.

It is worth mentioning that the intestine and the lungs showed more or less similar sequences of reaction to ova and parasites. Few parasites were destroyed in the intestine, but practically all of the many that reached the lungs were killed soon after their arrival. Extensive lesions throughout the intestine, due chiefly to ova, and in the lungs, due to parasites and ova, resolved in later stages so that scarcely an ovum could be found in numerous sections of either of these organs. This again was due both to destruction of parasites within the portal system and to massive migration of the worms away from it through portal-systemic anastomoses. Relatively few viable parasites remained, therefore, within the portal system in these late stages.

The spleen at the height of the infection revealed sinusoidal congestion, abundant parasitic pigment in follicles and pulp and very rarely a thrombosed sinus or parasitic ovum. Increase in reticulum, follicular fibrosis or siderosis in any way comparable to such changes in the human spleen with chronic passive congestion were never seen. In late stages the spleen was practically normal in all respects.

PORTAL-SYSTEMIC COLLATERAL VEINS

The time of appearance and the extent of development of the portal-systemic collateral veins were dependent on the degree of the infection, the capacity of the portal system, the size of the liver (as an index of the capacity of the intrahepatic portion of the portal venous system) and probably other factors, such as the viscosity of the blood, anemia and the systemic venous pressures. Well nourished animals with a more capacious intrahepatic and extrahepatic portal system required a somewhat longer period of oviposition before showing collateral pathways as compared with emaciated, anemic animals. Thus, collateral change is noted first between the forty-ninth and the seventy-eighth day in the former and after the fortieth day in the latter. The increase in their size or in their number or in both was

roughly proportionate to the increase in the extent and the degree of intrahepatic vascular obstruction. In the same way they decreased in size and/or in number with resolution of the lesions, though rarely completely, even in the very late stages of the infection. Generally at the height of the infection they were accompanied by dilatation of the extrahepatic radicles of the portal vein, best seen in the dilated and somewhat tortuous serosal veins of the intestine. Collateral veins were, however, present in the absence of such general dilatation of the portal system.

Portal-systemic anastomoses of relatively minor import were noted as follows: 1. There were prominent periesophageal veins which anastomosed with phrenic or intrathoracic veins, but anything even remotely approaching esophageal varices was never seen. 2. One or more small veins in the falciform ligament anastomosed with the superior epigastric or phrenic veins. 3. While the superior hemorrhoidal vein was often dilated and somewhat tortuous, we could never satisfactorily expose the middle and inferior hemorrhoidal veins, but here again anything even remotely indicative of anorectal mucosal or submucosal varices was never seen. 4. Arising from the dorsal venous arcade of the inferior mesenteric vein there was often a single-looped or double-looped branch which adjoined, or enclosed within the loop, a small lymph node from which it might receive efferent veins. This vein also received one or more small additional mesocolic veins and then, running fairly straight dorsally in close relation to the inferior mesenteric artery, reached the base of the mesocolon, where it often divided into a number of branches which entered either the inferior vena cava proper or the right or the left ureteral or the left or the right spermatic (ovarian) vein. Its finer divisions were often lost in the retroperitoneal tissues. In the normal guinea pig this branch of the inferior mesenteric vein was often seen with greater or lesser distinctness. Often it appeared interrupted in its ventral course, or its dorsal divisions were bloodless and untraceable. Rarely, even in the normal animal, it formed quite a prominent vein. Adult parasites were rarely seen in any of these minor anastomoses.

The major anastomoses occurred in two distinct regions: (1) the portocaval triangle and (2) the lienopancreatic-mesoduodenal area. With the liver reflected cranially and the stomach displaced to the left, the portocaval triangle was roughly formed by the liver at its apex, the portal and the superior mesenteric vein to the left, the inferior vena cava to the right, and an imaginary base line running between the dorsal loop of the duodenum and the right kidney, and the floor, by the retroperitoneum caudal to the foramen of Winslow. Within this space even in the normal guinea pig there are very delicate, barely visible veins which, arising from the pancreatic tissue dorsal to and about the portal and superior mesenteric veins, including the biliary lymph node, or from the fixed dorsal loop of duodenum, run retroperitoneally toward the inferior vena cava. These veins, however, run for short distances, are often interrupted and lost and are rarely seen to enter systemic veins. In the normal rat there is almost constantly a larger vein which arises most frequently in the region about and often probably from the inferior mesenteric vein, just before it joins the superior mesenteric vein, which runs retroperitoneally, generally emptying into the inferior vena cava or the right lumboadrenal confluence of veins. With portal hypertension, the veins in this triangle (fig. 2*A*) enlarged. At times they formed a leash of vessels, one or more of which became very large, often straight, at times mildly tortuous, almost comparable in size with the portal vein itself. These vessels were now more clearly seen to arise either from the superior mesenteric, gastrosplenic and gastroduodenal veins or from their smaller pancreatic or duodenal tributaries. The larger of these veins either singly or combined emptied either directly into the inferior vena cava, the left renal vein near its entrance into the vena cava or the right adrenal, the right lumbar or the right renal vein. The smaller veins were again often interrupted and untraceable.

By displacing the stomach and intestines to the right and by gently pulling on them ventrally, the spleen, the pancreas, the mesoduodenum and the mesocolon are exposed. In the normal guinea pig there are a few very delicate veins dorsal to the celiac axis and the superior mesenteric artery (in the guinea pig generally a direct offshoot from the celiac axis) the complete course of which it is difficult to trace. No veins could be recognized, however, in the mesoduodenum between the pancreas and the celiac axis (superior mesenteric artery). With portal hypertension, a leash of small and, as described in the foregoing paragraph, larger, often more tortuous veins appeared (fig. 2*B*). These arose from the gastrosplenic vein or its pancreatic tributaries and after running within the dorsal portion of the mesoduodenum and then to the left, retroperitoneally, either by division or by union, emptied singly or in various combinations into the left renal and renal peripelvic or the left adrenolumbar confluence of veins. In addition there were one or more veins which arose either directly from the inferior mesenteric vein close to its entrance into the superior mesenteric vein, from the latter itself or from its pancreaticoduodenal branches within the dorsal loop of the duodenum.

They pursued an irregular course, part of which could be seen in the portocaval triangle, which generally crossed the celiac axis (superior mesenteric artery) and ran parallel but dorsal to it, emptying either directly into the left renal vein, its adrenolumbar confluence or at times into the left spermatic (ovarian) vein. Finally there was often a vein which arose from the cranial branch of the splenic vein close to the hilus (or from smaller vasa brevia) which curved about the superior pole of the spleen, entered the lienophrenic ligament and, descending caudally, joined either the phrenic vein or the pancreatic collateral vessels, finally draining into the left adrenolumbar confluence of veins.

Parasites were often seen singly or in larger numbers within these major anastomoses. It is unlikely that these parasites were in any way instrumental in widening these established pathways; rather they used them as conveniently wide channels through which they could slip.

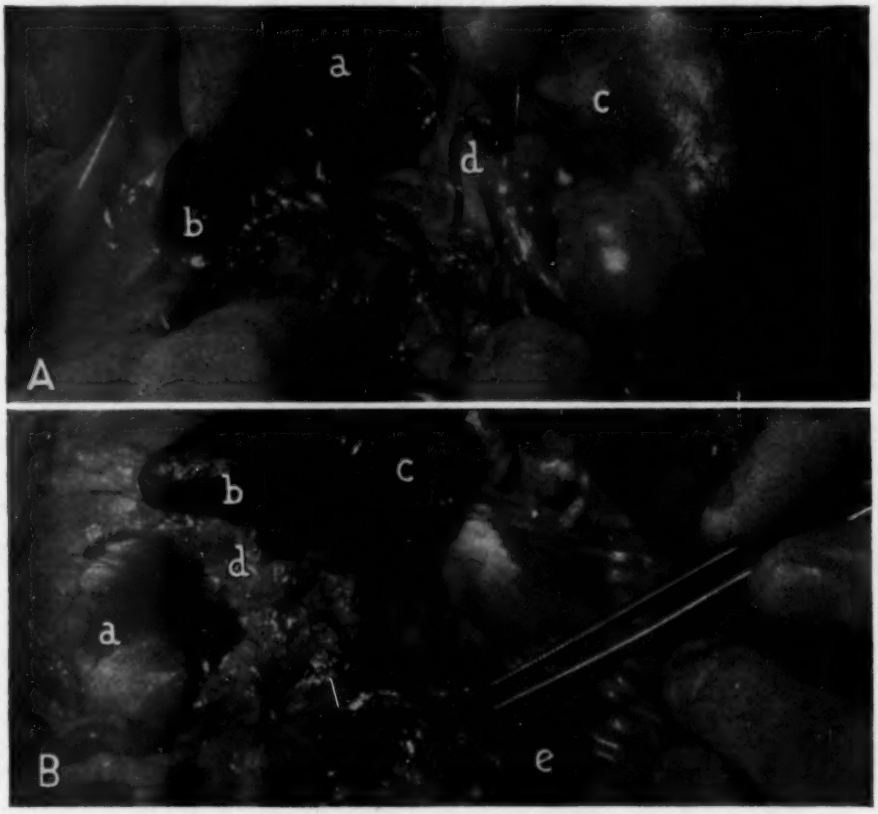

Fig. 2.—*A*, portocaval triangle: (*a*) liver, (*b*) right kidney, (*c*) stomach, (*d*) portal vein. To the left of the portal vein is the enlarged biliary lymph node. Between the latter and the inferior vena cava are some small retroperitoneal veins. Below these there is a large tortuous vein arising from the superior mesenteric vein and entering the bulb of the inferior vena cava. The tortuous anastomotic vein to the left of it is best seen in *B*, entering the left renal vein in conjunction with the left spermatic vein. *B*, lienopancreatic-mesoduodenal area: (*a*) stomach, (*b*) spleen, (*c*) liver, (*d*) pancreas, (*e*) left kidney. The forceps retracts the left adrenal. A vein partly obscured by fat runs from the cranial pole of the spleen to join the pancreatic collateral veins. Two of the latter it will be noted arise from a large branch entering the cranial branch of the gastrosplenic vein. These pancreatic collateral veins cross the celiac axis (superior mesenteric artery) and empty into the adrenolumbar confluence of veins. Below the left renal vein a tortuous anastomotic vessel runs transversely from the inferior mesenteric vein to join the left spermatic vein before entering the left renal vein close to the hilus of the kidney.

These major anastomoses, including those from the distal divisions of the inferior mesenteric vein, belong to the anastomotic veins of Retzius. One or more of these veins were

described at various times by Haller [10] (even before Retzius), Cruveilhier,[11] Claude Bernard [12] and Tuffier and Lejars,[12] while their constant and multiple character was first adequately described by Gilbert and Villaret,[2] who observed them in dogs and guinea pigs.

COMMENT

If we restrict the term "congestive splenomegaly" to the spleen weighing more than three times its normal weight (which is considered to be the limit of physiologic congestion) with widened, congested sinusoids, reticular fibrosis of the pulp, fibrosis of the splenic corpuscles and siderotic nodules, we find little reference to the condition in animals under either experimental or natural conditions. The literature on this subject has been recently reviewed by McMichael,[13] Menon [14] and Cameron and de Saram.[15] The authors named concluded that in the development of congestive splenomegaly two independent factors are probably involved, viz., hyperplasia of the splenic pulp and portal congestion. They failed to explain, however, the inability to reproduce these classic anatomic splenic changes in experimental animals. The schistosomal experiments reported here are further proof of this difficulty despite the presence of both factors: hyperplasia of the splenic pulp due to the storage of parasitic pigment and marked intrahepatic portal obstruction due to the destruction of parasites and the deposition of ova. Similarly, Fairley and Mackie [16] reported the absence of splenomegaly in goats infected spontaneously or experimentally with Schistosoma spindale, and Le Roux,[17] in sheep infected spontaneously with Schistosoma mattheei. Rousselot and Thompson,[18] however, reported great enlargement of the spleen in dogs following several injections of suspensions of silica into the splenic vein. This was accompanied by marked hepatic cirrhosis. Neither the weight of the spleen nor an adequate histologic description of the organ was given. Furthermore, no mention was made of the absence or the presence of pulmonary fibrosis resulting from such injections which might have raised the systemic venous pressure and thus influenced the enlargement of the spleen.

It is known that acute portal congestion produced experimentally, by narrowing the portal vein or, as in the classic experiments of Rous and Larimore,[5] by ligating the portal branch to the main lobe of the liver in the rabbit, while tying the branch of the bile duct to the posterior lobe mass, results in appreciable enlargement of the spleen. The collateral veins that develop become adequate in brief time, however, to relieve all portal congestion, with reduction of the spleen to normal size. Rousselot and Thompson [18] likewise stated that in their various attempts to produce experimental congestive splenomegaly by constriction of the splenic vein their results were "uniformly disappointing as either complete venous occlusion develops with splenic atrophy or an adequate collateral promptly forms with no alteration in the size of the spleen." It has been amply shown by these two investigators [19] that in human congestive splenomegaly there is portal or splenic venous hypertension. It is probable that the former is largely the result of the

10. Haller, cited by Stopford, J. S. B.: M. Chron. **61**:12, 1915.
11. Cruveilhier, cited by Duskes, E.: Arch. Surg. **15**:580, 1927.
12. Cited by Gilbert and Villaret.[2]
13. McMichael, J.: J. Path. & Bact. **39**:481, 1934.
14. Menon, T. B.: J. Path. & Bact. **46**:357 and 521, 1938.
15. Cameron, G. R., and de Saram, G. S. W.: J. Path. & Bact. **48**:41, 1939.
16. Fairley, N. H., and Mackie, F. P.: Indian M. Research Mem. **17**:17, 1930.
17. Le Roux, P. L.: Fifteenth Annual Report of the Director of Veterinary Sciences, Department of Agriculture, Union of South Africa, 1929.
18. Rousselot, L. M., and Thompson, W. P.: Proc. Soc. Exper. Biol. & Med. **40**:705, 1939.
19. Rousselot, L. M.: Surgery **8**:34, 1940. Thompson, W. P.: Ann. Int. Med. **14**:255, 1940.

latter. We should like to point out on theoretic grounds how adequate portal-systemic collateral veins could operate to produce lower portal venous tensions in small animals than in man, and hence contribute one reason, at any rate, for this difficulty in producing congestive splenomegaly experimentally.

First, in the experimental animal there is no appreciable hydrostatic pressure due to gravity to augment the existent pressure in the portal venous system. Second, in rodents there is no parietal peritoneal fixation of the pancreas, the duodenum and the colon (except for the pelvic colon), but in its place there is a dorsal mesoduodenum enclosing the diffuse pancreas, while common mesentery and mesocolon suspend the large bowel. This restricts the anatomic fields where portal-systemic anastomoses can readily be established. Likewise in larger mammals, while the duodenum and the pancreas are fixed, the colon generally is suspended from the common mesentery and mesocolon. Third, in the small experimental animal the capacity of the portal venous system is correspondingly small. It follows, therefore, that given a certain amount of time for collateral channels to become established, the short, quite direct, relatively few yet amply wide anastomoses between the larger radicles of the portal vein, such as the superior mesenteric and the gastrosplenic (where volume flow per minute is greater than in the peripheral parts of the portal system), and the large systemic vessels can shunt the smaller volume of portal blood more rapidly into the systemic veins, with the maintenance of less elevated portal pressures, provided the systemic venous pressure is not increased for one or another reason. As shown here, there is no necessity for creating new collateral pathways through operative interferences to obtain adequate diversion of blood flow. The effect is a little more like that of an Eck fistula, through which the whole portal stream can be diverted into the inferior vena cava without producing any appreciable degree of portal hypertension.

In man, on the other hand, with dorsal fixation of the duodenum, the pancreas and the colon there is, first, an extensive area of close contact between the small vessels of the portal system and those of the retroperitoneal tissues, where numerous small anastomoses can be established. Second, there are rarely direct major anastomoses between the superior mesenteric or the splenic vein and veins of the systemic system. Third, the splenic vein is fixed in position and hence is presumably less distensible than is that in the diffuse pancreas of the rodent. Fourth, there is greater portal venous capacity [20] and hence a larger volume of blood that has to be shunted away from the obstructed region. In view, therefore, of the greatly widened cross-sectional bed created by numerous large and small anastomoses with superimposed increased frictional factors, it might be expected that the outflow of blood distal to the portal obstruction would be slower and the tension accordingly more elevated; i. e., even with adequate collateral channels, the excess volume of portal blood is diverted only at the expense of higher portal pressures. Fifth, there is added the hydrostatic factor of man's upright posture, which tends to produce far higher portal and splenic venous pressures than are found in lower animals.

As demonstrated here, the collateral veins are labile structures, and with concentration of blood flow along one or other established pathway there may be diminution in size or disappearance of other, less favored channels. The same flux in number and size of collateral veins probably occurs in man; i. e., there may be

20. This would include any return flow from the hepatic artery into the portal vein, as demonstrated by F. C. Herrick (J. Exper. Med. **9**:93, 1907) in human portal cirrhosis, and digestive engorgement of the portal venous system, which has been stressed by E. Jäger (Virchows Arch. f. path. Anat. **299**:531, 1937) as imposing work hypertrophy and therefore progressive enlargement of the spleen in man. Both are applicable to animal forms.

diffuse collateral pathways of small and larger size, particularly in the earlier stages, during the development of portal obstruction, which may remain more permanently, or other vessels may open up and take on the greater volume of outflow with diminution in number and size of previously established collateral veins. While, therefore, many small and larger retroperitoneal anastomoses would favor higher portal pressures, according to this view, larger and fewer collateral veins, which might for a time act more efficiently in relieving portal congestion, would by virtue of the large volume of blood forced through them at higher pressures and the weakness of their walls give rise to greatly widened tortuous and lengthened varicosities in which the slowing of blood flow would again operate in raising the portal pressure. Splenomegaly, therefore, in association with great widening and tortuosity of paraumbilical veins in the Cruveilhier-Baumgarten syndrome or in those few recorded instances in which large tortuous varicose veins connected splenic and renal venous systems (Rössle [21]; Ligars [21]; Mariau [21]; Löffler [21]; Saxer [22]; Trimble and Hill [23]) is as such a little more understandable.[24]

These remarks apply generally to congestive splenomegaly associated with intrahepatic portal obstruction as well as to obstruction of the portal vein itself. With obstruction or compression of the splenic vein, the larger outflow of blood from the spleen in man, accompanied more frequently by inadequacy and subsequent varicosity of collateral veins, would tend to produce greater elevation in splenic venous pressure. On the other hand, in small experimental animals the smaller volume of blood that has to be diverted and the ready establishment of major collateral paths from the gastrosplenic vein would again tend to modify any hypertension.

While it therefore seems understandable how small laboratory animals could cope more efficiently with portal congestion without acquiring splenomegaly or necessarily esophageal, epigastric or hemorrhoidal varices, with regard to larger animals there is need for further information on the pattern and the character of portal-systemic collateral veins and the occurrence of congestive splenomegaly as here defined. In these larger animals, except for hydrostatic and other anatomic factors, such as lack of parietal fixation of the colon, equally large or even larger volumes of blood as compared with that in man would have to be diverted in either natural or experimentally induced portal obstruction. There is need too for more information on the height of portal blood pressure required to produce progressive distensibility of the spleen. It is possible that the small spleens of laboratory animals require much higher pressures to produce enlargement beyond physiologic limits than the larger spleens of higher forms (Cook [25]). The lesser degree of splenomegaly noted in older persons with portal obstruction (Johnston [26]) may be due in part to the original atrophic and fibrotic changes in the spleen in

21. Cited by Jäger, E.: Virchows Arch. f. path. Anat. **299**:531, 1937.
22. Saxer, F.: Centralbl. f. allg. Path. u. path. Anat. **13**:577, 1902.
23. Trimble, W. K., and Hill, J. H.: Arch. Path. **34**:423, 1942.
24. Since the flow through collateral channels is entirely dependent on the differential pressures in portal and systemic veins, any rise in systemic venous pressure will be an important factor in further augmenting portal pressure and may lead to complete decompensation of the portal circulation. Lowered systemic venous pressures would have an opposite effect. The vertebral venous plexus, as stressed by O. V. Batson (Ann. Int. Med. **16**:38, 1942), serves as a low pressure reservoir with which the portal collateral veins may communicate. Efficient connections between these two systems may help to lower portal hypertension appreciably. It may be that the lesser degree of splenomegaly in older persons with portal obstruction may be partly ascribed to this greater efficiency (see text).
25. Cook, D. H.: Personal communication to the author.
26. Johnston, J. M.: Ann. Int. Med. **4**:772, 1931.

these persons requiring even higher splenic venous pressures than that generally obtainable. Inasmuch as a prolonged time is involved in the clinical development of congestive splenomegaly (Snell [27]; Larrabee [28]), this together with the degree of obstruction would have to be taken into account in experimental observations of this kind.

These portal-systemic collateral veins are of particular importance in schistosomiasis, for they serve as convenient pathways through which parasites, ova and parasitic pigment can be diverted from the portal system, particularly to the lungs, thus serving to limit continued damage to the liver and the intestine. In the guinea pig with a relatively small portal capacity and with the parasites therefore more limited in their migrations, a single heavy infection produces extensive intrahepatic portal obstruction, which with the establishment of collateral veins permits extensive and at times even massive parasitic migration. It is worth noting in this connection to what extent, with reduction of the parasitic burden in the portal system, repair and restitution to integrity can occur in the liver and the intestine and subsequently in the lungs. This is in conformity with the observations of Bollman and Mann,[29] Bollman [30] and Cameron and Karunaratne,[31] who reported that experimental hepatic cirrhosis is reversible provided it has not reached such a stage of fibrosis with vascular alteration as to render reversibility unlikely.

Similar factors are evidently operative with Schistosoma mansoni infections in man but are modified by the greater capacity of the portal system, the greater preference of the parasite for the ileocolic and colic veins and the occurrence of repeated infections. However, the site, the size and the number of collateral veins and their relation to those parts of the portal system most densely infected by the parasites at any one time will determine the degree of migration of the worms. The diversion of ova into extraportal sites will be very much less dependent on such close relationship for, being embolic, they will drift with the portal stream craniad from the site of their release and may therefore enter anastomoses distant from the parasitic habitat. Mainzer's [32] description of roentgenologic changes in the lungs ninety days after the known onset of S. mansoni infections is in agreement with our observations as to the onset of parasitic migration and diversion of ova from the portal system a short while after oviposition has set in and sufficient intrahepatic obstruction has been produced to call forth collateral veins or to help enlarge those already existent, such as the hemorrhoidal veins.[33] The paucity of parasites in human beings with advanced schistosomal cirrhosis of the liver is also an indication of the number of these parasites which have migrated and been destroyed in extraportal sites, in addition to those destroyed in the portal system proper. An index of this migration and diversion is also given by the many extraportal sites where ova have been found to be deposited.

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27. Snell, A. M.: Ann. Int. Med. **5**:338, 1931.
28. Larrabee, R. C.: Am. J. M. Sc. **188**:745, 1934.
29. Bollman, J. L., and Mann, F. C.: Ann. Int. Med. **5**:699, 1931
30. Bollman, J. L.: Proc. Staff Meet., Mayo Clin. **11**:727, 1936.
31. Cameron, G. R., and Karunaratne, W. A. E.: J. Path. & Bact. **42**:1, 1936.
32. Mainzer, F.: Puerto Rico J. Pub. Health & Trop. Med. **15**:111, 1939.
33. It is readily understandable that the collateral veins must be wide enough to admit freely parasites or ova probably a millimeter or more in diameter. If they are too narrow they are apt to be obstructed by these foreign bodies and the subsequent reaction to them. Small retrocolic anastomoses in man may therefore become obstructed, entailing greater difficulties in the escape of parasites and in the establishment of collateral channels. Of the other common large anastomoses, the paraumbilical veins, because of their direct connections with the left branch of the portal vein, would be more important channels for diversion of parasites and ova than the esophageal veins through their connections with those of the gastric veins.

With regard to human infections with Schistosoma haematobium, it is known that there is a long prepatent period before ova appear in the urine. In experimental S. haematobium infections of monkeys, however, Leiper [34] and Brumpt [35] found the adult parasites only in the portal venous system. Brumpt [35] reported the same findings in a number of small laboratory animals. Fairley,[36] on the other hand, obtained characteristic lesions in the urinary bladder in monkeys comparable to those which occur in man. These differences become clear when it is recalled that in quadrupeds, in the practical absence of hydrostatic pressure, the hemorrhoidal complex is less apt to become enlarged as it is in man. An index thereto, including factors of constipation, increase in intrarectal pressure for other reasons, or disposition and existence of valves in the portal venous system (Reuther [37]), is the rarity of hemorrhoids in nonhuman forms. Hutyra and Marek [38] reported the presence of hemorrhoids in dogs and horses only. The sequence of events in human infections with S. haematobium is therefore like that in infections with S. mansoni in its early stages with the difference that after oviposition has set in and a certain amount of intrahepatic obstruction has been produced the anastomoses between superior, and middle and inferior hemorrhoidal veins enlarge. This, together with a marked tropism for the genitourinary tract exhibited by this species of parasite (Brumpt [35]), permits more extensive and slow migration of the adult parasites to the walls of the urinary bladder by way of the hemorrhoidal veins through the internal pudendal and hypogastric veins. The long prepatent period is thus explained, as are the minimal lesions in the liver and the intestine in pure S. haematobium infections. In laboratory animals with lack of appreciable enlargement of the hemorrhoidal complex of veins but with development of efficient collateral channels in the mesoduodenal region, the parasites tend to be retained within the portal system. In migrating they are too distant from the lower genitourinary tract and, too, are more apt to be swept toward the heart and the lungs by the more rapid current in the larger systemic vessels anteriorly. To the extent that the hemorrhoidal anastomoses enlarge with age (Reuther [37]) and at the same time are dependent on posture, it is possible that older animals that assume a sitting position for long periods (e. g., monkeys) may develop a sufficiently widened anastomotic system in this region to explain Fairley's observations.

SUMMARY

The portal-systemic collateral veins which develop in the guinea pig experimentally infected with Schistosoma mansoni are described. For anatomic and physiologic reasons, the view is expressed that in laboratory animals at any rate such anastomoses may be expected to function more efficiently in maintaining lower portal (splenic) venous pressures in the presence of factors leading to portal (splenic) venous hypertension than might be expected in man. To the extent that congestive splenomegaly is related to portal (splenic) venous hypertension, the failure to reproduce this condition experimentally is more readily understandable. The importance of these anastomoses in schistosomiasis is stressed inasmuch as they serve as convenient pathways through which the parasites, their pigment and their ova can escape from the portal venous system.

34. Leiper, R. T.: Researches on Egyptian Bilharziosis, London, John Bale Sons and Danielsson, Ltd., 1918.

35. Brumpt, E.: Précis de parasitologie, Paris, Masson & Cie, 1936.

36. Fairley, N. H.: J. Path. & Bact. **23**:289, 1920.

37. Reuther, T. F.: Am. J. Surg. **49**:326, 1940.

38. Hutyra, F., and Marek, J.: Special Pathology and Therapeutics of the Diseases of Domestic Animals, Chicago, Alex Eger, 1926, vol. 2.

CALCIFICATION OF THE BONE MARROW IN TOXIC HYPERPARATHYROIDISM

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So-called metastatic or pathologic calcification has been demonstrated in many organs of human bodies coming to necropsy after prolonged periods of parathyroid hyperactivity,[1] as well as in many experimental animals following repeated parenteral injections of parathyroid extract.[2] Barr [3] recently reviewed the subject of pathologic calcification, making repetition unnecessary here. Pathologic calcification of marrow has not been reported in either clinical or experimental hyperparathyroidism.

McLean and Bloom,[4] in recent studies of puppies and young rats that were subjected to massive doses of solution of parathyroid U. S. P., have demonstrated calcium deposits in the marrow spaces of these animals as early as twenty-four hours after a single massive dose in the former and at forty-eight hours after repeated large doses in the latter. Evidence of abnormal absorption of metaphysial bone was present after eight to twelve hours in all such animals, and the calcium deposits were interpreted as precipitations in the process of transportation of bone salt from the trabeculae to the blood stream. The localization of the precipitates was ascribed to the presence of bone substrate which had been absorbed simultaneously with the salt and which from undetermined causes had become recalcifiable while lying free in the interstices of the marrow. Intracellular deposits, observed later, were attributed to phagocytosis of particles of the recalcified substrate by macrophages of the marrow.

Focal necrosis of elements of marrow as well as of tissues of other organs, including the liver, the spleen, the thymus, the heart and the kidneys, of the puppies was observed, but calcium deposits were not seen in tissues stained with hematoxylin and eosin.

From these findings and from personal observations (unpublished) of pathologic changes in bones of animals treated with less toxic doses of solution of parathyroid U. S. P. which showed no necrosis or calcification of marrow, it was felt that the widespread focal degeneration of parenchymatous organs was of significance in view of the fact that calcification of marrow occurred with similar focal necrosis. The following experiment was therefore undertaken to determine whether calcium

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Eli Lilly & Company furnished a substantial portion of the solution of parathyroid U. S. P. used in the present work.

1. Barr, D. P., and Bulger, H. A.: Am. J. M. Sc. **179**:449, 1930. Dawson, J. W., and Struthers, J. W.: Edinburgh M. J. **30**:421, 1923,

2. (*a*) Cantarow, A.; Stewart, H. L., and Housel, E. L.: Endocrinology **22**:13, 1938. (*b*) Hueper, W.: Arch. Path. **3**:14, 1927. (*c*) Learner, A.: J. Lab. & Clin. Med. **14**:921, 1929. (*d*) Leberman, P. R.: Surg., Gynec. & Obst. **70**:925, 1940. (*e*) McJunkin, F. A.; Tweedy, W. R., and Brehaus, H. C.: Arch. Path. **14**:649, 1932. (*f*) Shelling, D. H.; Asher, D. E., and Jackson, D. A.: Bull. Johns Hopkins Hosp. **53**:348, 1933. (*g*) Shelling, D. H.: The Parathyroids in Health and Disease, St. Louis, C. V. Mosby Company, 1935.

3. Barr, D. P.: Physiol. Rev. **12**:593, 1932.

4. McLean, F. C., and Bloom, W.: Arch. Path. **32**:315, 1941.

was deposited in other tissues under identical conditions, suggesting, if it was, that the calcification of marrow is a manifestation of generalized metastatic calcification rather than of precipitation of salt due to local circumstances of a unique nature at the site of mobilization of bone salt.

EXPERIMENT

The experimental data are summarized in the accompanying table.

A litter of 6 white rats, 29 days of age, was selected and maintained on a stock diet, fresh greens and water ad libitum during the course of the experiment. One of the litter was killed as the control. The remaining 5 animals were given, by intraperitoneal and subcutaneous routes alternately, solution of parathyroid U. S. P. in doses of 100 units twice daily. After forty-eight hours all of the animals showed toxic symptoms, consisting of ataxia, lassitude, cyanosis, diarrhea and frequent tremors. Rat A1, killed at forty-eight hours, showed mild early toxic symptoms after receiving 400 units. None of the remaining animals was alive after sixty-four hours.

For microscopic examination, blocks of tissue were taken from a tibia, a femur, a kidney, the heart, the liver, the lungs and the brain. The soft tissues were fixed in neutral 80 per cent alcohol and Helly's fluid. The material fixed in 80 per cent alcohol was prepared for studies of calcification by the Gömöri technic, and those fixed in Helly's fluid were decalcified in 6 per cent salicylic acid, embedded in nitrocellulose and stained with hematoxylin and eosin. Bone tissues were fixed in 80 per cent alcohol, neutral 4 per cent solution of formalde-

Experimental Data

Rat	Weight, Gm.		Units Solution of Parathyroid U. S. P.	Time of Death	Calcification						
	Onset	End			Marrow	Kidney	Heart	Stomach	Liver	Brain	Lungs
A0	37.5	....	0	Killed 0	0	0	0	0	0	0	0
A1	43.0	41.5	400	Killed 48 hr.	+	+	0	0	0	0	0
A2	44.0	42.5	500	Died 56-64 hr.	+	+	+	+	0	0	0
A3	43.5	41.7	500	Died 56-64 hr.	+	+	+	0	0	0	0
A4	43.0	41.5	500	Killed 52 hr.	+	+	+	0	0	0	0
A5	45.0	44.5	500	Killed 51 hr.	+	+	+	0	0	0	0

hyde and Helly's fluid and stained by Gömöri's calcium method, von Kossa's technic, hematoxylin and eosin, and hematoxylin-eosin-azure II.

GROSS OBSERVATIONS

Examination of rat A1, killed at forty-eight hours, showed pinpoint white mottling of the external surface of the kidneys with occasional larger pale areas. The stomach was moderately distended with fluid and undigested food. The epiphyses of the long bones separated more easily than normal, and the bones were softer in consistency than those of the control. The remaining 4 animals, 2 of which died and 2 of which were killed between fifty-one and sixty-four hours after the start of treatment, all showed marked renal mottling with pallor of the central zone on cut section and similar mottling of the heart muscle with distinct larger white areas of infarction. All showed marked gastric distention and epiphyses which separated under little stress. Periosteal hemorrhages were noted in the 2 animals dying between fifty-six and sixty-four hours.

HISTOLOGIC OBSERVATIONS

The tibia of rat A1, killed at forty-eight hours, showed marked absorption of the primary substantia spongiosa and fibroblastic proliferation of the marrow, which contained many multinucleated giant cells (fig. 1*C*). The epiphysial cartilage plate was separated from the metaphysis in places by cystic spaces partially filled with fibrin and cellular debris. Throughout the zone of contact of epiphysial plate and metaphysis were considerable necrosis and liquefaction of proliferating fibrous tissue. Deeper in the substantia spongiosa, focal areas of necrosis of marrow were observed, and isolated giant cells frequently exhibited pyknotic nuclei and cytoplasmic dissolution.

Calcium stains showed simultaneous disappearance of bone or calcified matrix of cartilage and salt but no evidence of phagocytic activity either on the part of mononuclears or on that

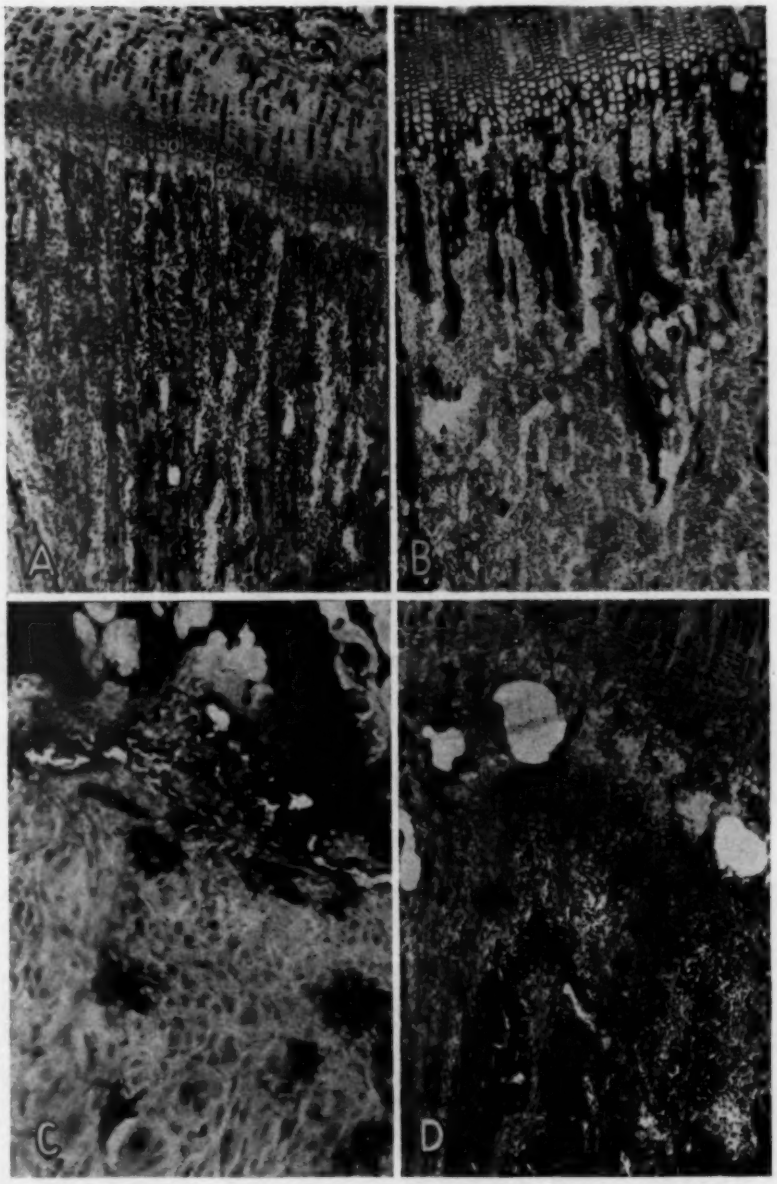

Fig. 1.—*A*, epiphysial cartilage plate and substantia spongiosa of normal rat A0, showing orderly enchondral ossification. Hematoxylin and eosin stain; × 80. *B*, epiphysial cartilage plate and substantia spongiosa of normal rat A0. Von Kossa calcium stain; × 80. Tissues containing calcium phosphate stain black. Note the absence of "osteoid" borders. *C*, epiphysial cartilage plate and substantia spongiosa of rat A1 after forty-eight hours of treatment with solution of parathyroid U. S. P. (given 400 units) showing cystic degeneration throughout the primary substantia spongiosa with fibrous tissue proliferation and giant cell formation. Hematoxylin and eosin stain; × 80. *D*, junction of calcified cartilage and primary substantia spongiosa of rat A1 after forty-eight hours of treatment, showing fibrous replacement of marrow with absorption of calcified cartilage and early calcification in the fibrous marrow of the substantia spongiosa. Gömöri's calcium stain; × 350.

of multinucleated giant cells. Near the epiphysial plate, in the zone of most marked degeneration, noted previously, were small deposits of calcium, appearing in stellate or rounded masses and smaller slender crystals (fig. 1 *D*). These were distinct from primary trabeculae and calcified cartilage columns undergoing absorption, which retained axial alinement and uniform density, with relatively smooth borders.

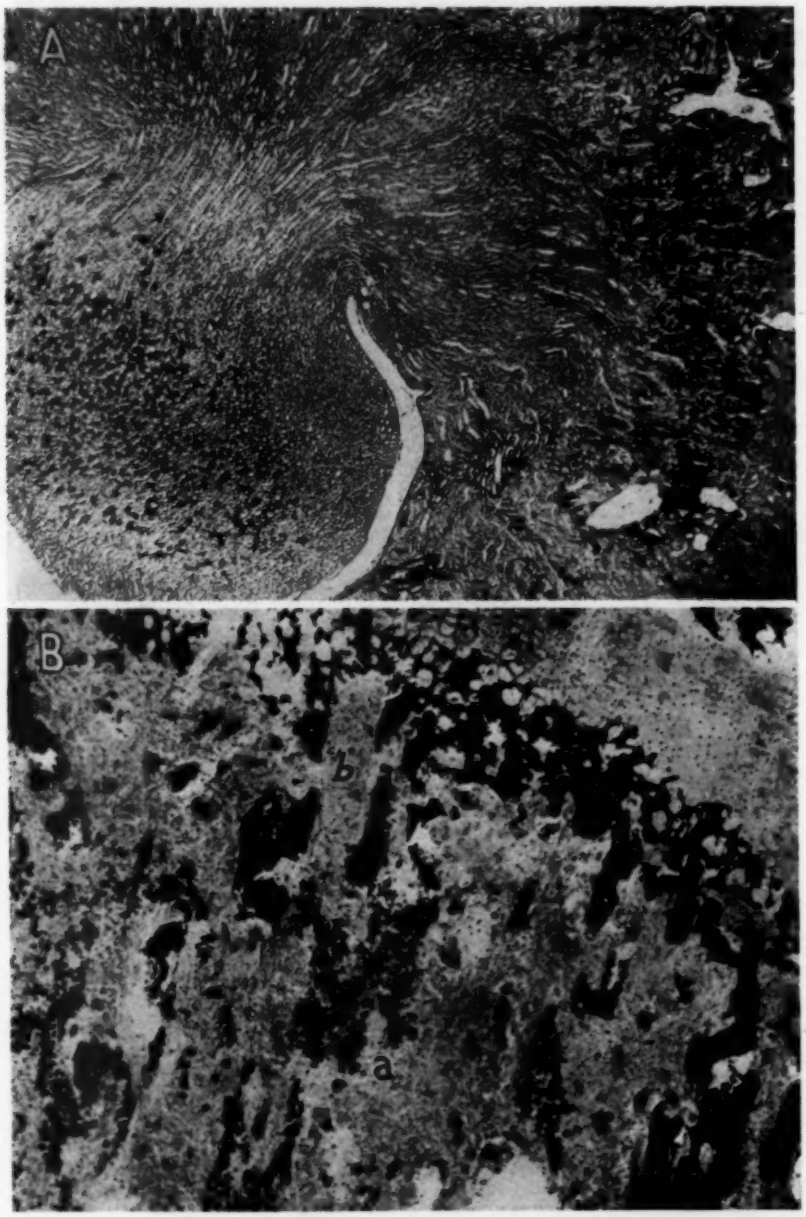

Fig. 2.—*A*, kidney of rat A1 after forty-eight hours of treatment, showing extensive calcification of the convoluted and collecting tubules. Gömöri's calcium stain; × 55. *B*, distal end of femur of rat 5, killed after fifty-one hours of treatment, showing marked absorption of primary substantia spongiosa with fibrous tissue replacement of the marrow and diffuse granular deposits of calcium (*a*). Unresorbed trabeculae and calcified cartilage (*b*) remain as dense black masses. Von Kossa's stain; × 140.

The kidney of rat A1 showed patchy tubular degeneration, with calcification within the epithelial cells of the convoluted and collecting tubules, the interstitial spaces and Bowman's capsule and as distinct tubular casts (fig. 2*A*). Evidence of early proximal tubular and glomerular distention was likewise noted. Other organs revealed no abnormalities.

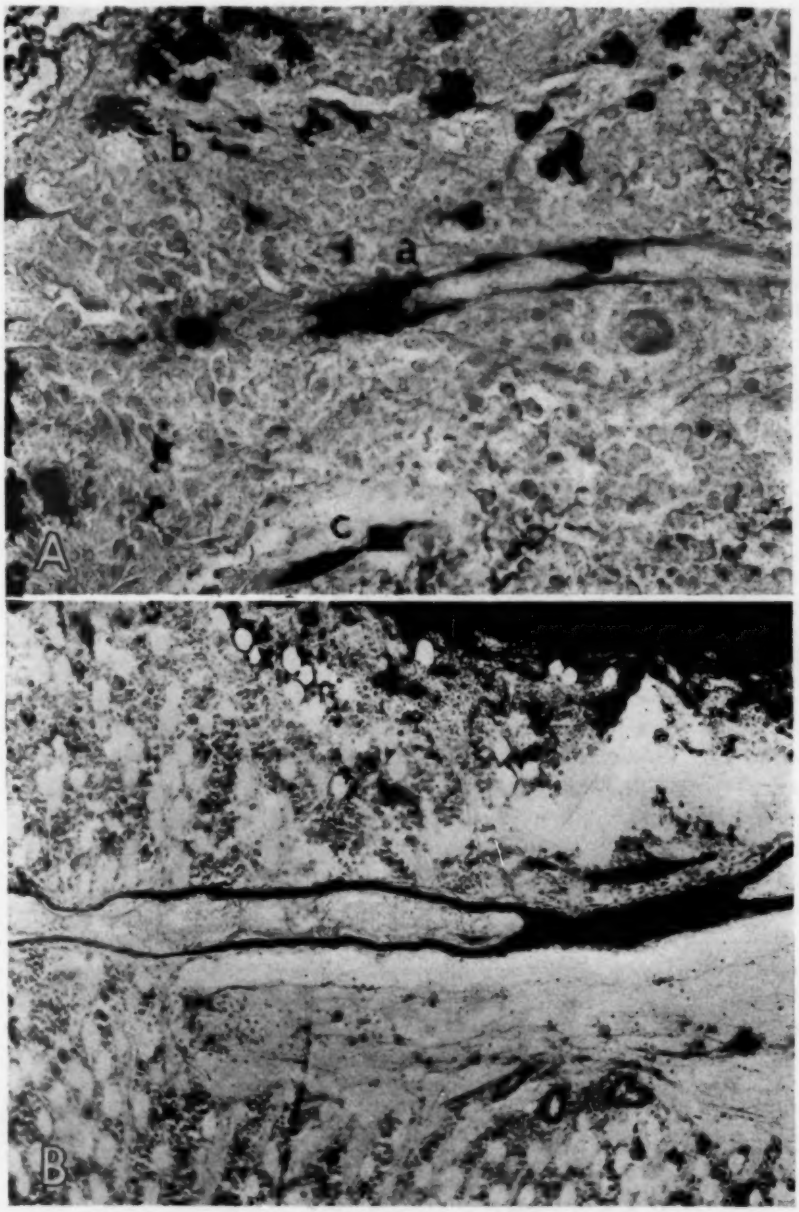

Fig. 3.—*A*, calcified arteriolar wall (*a*) in substantia spongiosa of rat A5. Note the granular deposits of calcium in the marrow (*b*) and unresorbed trabeculum (*c*). Gömöri's calcium stain; × 420. *B*, calcified nutrient artery in a diaphysis of rat A 4, killed after fifty-two hours of treatment. Note the deposits of calcium in the marrow of the endosteal zone of fibrous proliferation above and the absence of calcification in the hemopoietic marrow below Gömöri's stain; × 120.

The remaining animals of the series which were killed or which died between fifty-one and sixty-four hours showed more advanced areas of necrosis and calcification in the juxta-epiphysial region (fig. 2 *B*). The kidneys all showed extensive tubular degeneration with calcification as described in the foregoing paragraph and calcification of the walls of many small blood vessels. The cardiac muscle showed extensive calcification of the coronary vessels, with adjacent areas of calcified necrotic muscle fibers. The stomach of rat A2, which died within fifty-six to sixty-four hours, showed calcification within the gastric wall. Here the calcium was intracellular as well as extracellular and was deposited in the submucosa as well as diffusely throughout the mucosa. Focal degeneration of mucosal cells was noted in this instance but was not observed in the other animals. The livers of rats A2 and A3 showed patchy perivascular necrosis without calcification.

Calcification of vascular walls as observed in the kidneys and the cardiac muscle was observed in small arterioles of the metaphysis as seen in figure 3 *A*. Extensive calcification of the nutrient artery of rat A4, killed at fifty-two hours (fig. 3 *B*), was noted in the diaphysis, although extensive calcification of marrow was seen only in the metaphysial zone of fibroplasia and along the endosteal surface where similar proliferation occurred.

COMMENT

Thus, focal degeneration of tissues was noted in the kidneys, the stomach, the heart, the liver and the marrow of these animals. In each instance, except for the liver, calcification was observed when necrosis was demonstrable. Conversely, calcification was not observed where degenerative changes could not be demonstrated, including the stomach and the kidneys. In the latter, as was early suggested by Askanazy [5] and Hofmeister,[6] factors such as local alkalinity in tissues secreting acid substances may be the precipitating factor. In this series of animals such an explanation does not appear to be true, as calcification was not observed in the absence of necrosis.

Barr [3] distinguished between dystrophic calcification, which occurs locally following degenerative changes, and metastatic calcification, which occurs widely throughout the body in many bone and renal diseases in which local necrosis of tissues is not a precipitating factor. The reported cases of diffuse calcification found at autopsy in a person with osteitis fibrosa cystica have been included in this category because of the reported absence of degeneration of tissues at the sites of calcium deposition. It is well known, however, that the kidneys in such cases show progressive failure throughout the disease and that uremic death ensues. It is possible that calcification in other organs occurs in association with cellular damage during premortal toxic phases of the disease and that sufficient healing occurs before death to make detection of the focal damage of tissue difficult.

In adult rats subjected to toxic doses of solution of parathyroid, McJunkin, Tweedy and Brehaus [2e] found pathologic calcification limited to tissues showing necrotic changes. They suggested that necrosis is primary, always preceding the calcification. They found no evidence of calcification in tissues otherwise normal. Hypercalcemia and hyperphosphatemia were consistently present. Similar associated factors have been found in rats under the influence of toxic doses of viosterol by Shohl, Goldblatt and Brown,[7] in which focal changes of tissues always accompanied calcification in the presence of elevated levels of serum calcium and inorganic phosphorus.

Interpretation of the histologic alterations from the standpoint of the chemical changes in the blood in toxic states is difficult because of variation in the sensitivity of various animals to the solution of parathyroid. Rats, unlike dogs, tend to acquire

5. Askanazy, M.: Beiträge zur Knochenpathologie, in Chemischen und medicinischen Untersuchungen. Festschrift für Jaffe, Braunschweig, Friedrich Viewig & Son, 1901.

6. Hofmeister, F.: Ergebn. d. Physiol. **10**:429, 1910.

7. Shohl, A. T.; Goldblatt, H., and Brown, H. B.: J. Clin. Investigation **8**:505, 1930.

immunity to repeated doses of the solution after ten to twelve days.[8] However, Shelling, Asher and Jackson [2f] showed that toxic phases in rats are accompanied by hypercalcemia and hyperphosphatemia as in dogs.

In dogs repeated toxic doses of parathyroid extract [9] led to an early rapid rise in serum calcium, which attained a maximum in twenty to twenty-four hours, and remained at maximum until approximately the thirty-sixth hour, when a steady decline began. The urinary excretion of calcium, phosphorus and nitrogen increased soon after the onset of the experiment, and fecal excretion of calcium increased as well. After the twenty-fourth hour the blood nonprotein nitrogen and urea nitrogen began to rise and continued to rise until the end. Plasma inorganic phosphorus, which initially decreased slightly, later increased along with the rise in blood urea; hence it is likely that both rose as the result of progressive renal failure, as the animals rapidly became oliguric and urinary excretion of phosphorus fell rapidly.

Toxic symptoms appear at the time the serum calcium begins to fall and, as suggested by Thompson and Collip,[10] the fall in serum calcium is probably due to precipitation of calcium phosphate compounds, the solubility product of which is exceeded by the rising serum phosphorus.

Other contributing factors, including dehydration and loss of sodium and chloride, with progressive diminution of blood and plasma volume and increase in osmotic pressure, have been demonstrated by others.[11] Albright, Bauer, Ropes and Aub [12] demonstrated similar early changes following administration of a parathyroid extract to persons on a low intake of calcium.

The clinical course and the histologic appearance of the tissues of the animals reported here suggest a similar course of events under the condition described. It is felt that the calcium deposits in the marrow are of the same nature as those occurring in other tissues of the body and that the precipitates of calcium phosphate observed in association with focal necrosis of tissue are due to local establishment of concentrations of these ions in excess of their solubility product constant. Factors responsible for the localization of salt deposits in the presence of systemic hypercalcemia and hyperphosphatemia may consist of local release of intracellular phosphate incident to cellular degeneration or of changes in the alkalinity of tissue, from the same cause.

SUMMARY

Pathologic calcification of bone marrow in young rats subjected to toxic doses of solution of parathyroid U. S. P. is reported. The deposits of calcium phosphate in the marrow were in every instance associated with similar deposits in other organs. The salt precipitates were invariably accompanied by focal degeneration of the tissues involved, and no calcification of tissues otherwise normal was observed. The localization of the deposits appear to be induced by focal changes incident to cell degeneration in association with systemic hypercalcemia and hyperphosphatemia.

8. Pugsley, L. I., and Selye, H.: J. Physiol. **79**:113, 1933.
9. Collip, J. B.; Clark, E. P., and Scott, J. W.: J. Biol. Chem. **63**:439, 1925. Collip, J. B., and Clark, E. P.: ibid. **64**:485, 1925.
10. Thompson, D. L., and Collip, J. B.: Physiol. Rev. **12**:309, 1932.
11. (*a*) Cantarow, A.; Brundage, J. T., and House, E. L.: Endocrinology **21**:368, 1937. (*b*) Shelling, D. H.; Kajdi, L., and Goth, L.: ibid. **22**:225, 1938. (*c*) Thompson and Collip.[10]
12. Albright, F.; Bauer, W.; Ropes, M., and Aub, J. C.: J. Clin. Investigation **7**:139, 1929.

A GENETIC ANALYSIS OF THE INDUCTION OF TUMORS BY METHYLCHOLANTHRENE

V. ABSENCE OF SEX INFLUENCE WHEN A LARGE DOSE OF A CARCINOGEN IS ADMINISTERED

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Data on the role of sex in the induction of tumors by carcinogens are not in complete agreement. For example, Reinhard and Candee [1] and also Kreyberg [2] reported a longer latent period before tumor formation in males. On the other hand, Andervont [3] reported a longer latent period in females. Andervont used the C_{57} strain but expressed the opinion that this finding should not be accepted unreservedly. Sall and Shear [4] and Shear and Leiter [5] found a greater incidence of induced tumors in males (59 per cent) as contrasted with females (41 per cent) when minimal amounts of benzpyrene had been used (0.1 mg.). In another experiment, in which 0.08 mg. of benzpyrene was used, a similar sex difference was reported. They did not obtain a sex difference, however, when larger amounts were used (1 mg. per mouse). These authors expressed the opinion that perhaps the larger doses of carcinogen mask the possible effect of minor contributions, such as sex. The co-carcinogenic effect of sex and other factors was seen only when minimal amounts of carcinogen had been employed. Burdette and Strong [6] have shown that with the use of 1 mg. of methylcholanthrene there was no sex difference in the induction of subcutaneous tumors in the C_3H, CBA, NH and JK strains. In CHI mice, however, with the same dose there was a slight difference of twenty-three days in average induction time between the sexes. Here the difference in the median figures was eight days. Although not demonstrated conclusively, this sexual difference is suggestive and should be investigated further.

In the present study it is purposed to present data bearing on the possible role of sex in the induction of tumors by subcutaneous injection of 1 mg. of methylcholanthrene dissolved in 0.1 cc. of sesame oil. For this purpose two experiments dealing with the use of mice of the NHO strain are given.

MATERIALS AND METHODS

All of the mice included in this report were of the NHO strain. The origin of the NH strain has been discussed recently.[7] Briefly stated, it is as follows: The NH strain was originally established as a selective group of mice following tandem crosses between mice of

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This experiment has been made possible by grants from the Anna Fuller Fund and the Jane Coffin Childs Memorial Fund for Medical Research.

1. Reinhard, M. C., and Candee, C. F.: Am. J. Cancer **16**:640, 1932.
2. Kreyberg, L.: The Genetic and Constitutional Aspects of Spontaneous and Induced Tumors: A Symposium on Cancer, Madison, Wis., The University of Wisconsin Press, 1938, p. 3.
3. Andervont, H. B.: Pub. Health Rep. **53**:1647, 1938.
4. Sall, R. D., and Shear, J. S.: J. Nat. Cancer Inst. **1**:45, 1940.
5. Shear, M. L., and Leiter, J.: Unpublished data; cited by Sall and Shear.[4]
6. Burdette, W. J., and Strong, L. C.: Cancer Research **3**:13, 1943.
7. Strong, L. C.: Am. J. Cancer **39**:347, 1940.

the CBA, N and JK strains.[8] The mice give rise rarely to spontaneous tumors of various tissues, such as carcinoma of the lung, adenocarcinoma of the mammary gland and leukemia. One case of adenomatous hyperplasia of the stomach at 710 days of life (male mouse 152753) has been found. As a general rule, however, the strain must be considered very resistant to spontaneous tumor formation. The percentage incidence of all tumor types grouped together is well under 1.

In one subline of the NH strain stenosis of the esophagus is of frequent occurrence.[9] The different mice show the genetic characters, in various combinations, of black eye and pink eye, brown pigmentation of the hair, nonagouti, short and long ear, piebald spotting and self coloration.

In the early generations (F_5-F_8) following the establishment of the NH strain, groups of mice at 60 days of life were given by subcutaneous injection 1 mg. of methylcholanthrene dissolved in 0.1 cc. of sesame oil. The first paper in this series [7] gave data bearing on the conclusions (*a*) that specific types of tumors were induced and (*b*) that the specific tumor type responses tended to be transmitted to the direct descendants. The latter result was obtained by the application of the well known progeny test, that of continuing the descendants of the mouse, in each generation, which conformed to type.

Thus from the original tandem crosses referred to in the first paragraph of this section two sublines were established: (1) the unselected NH and (2) the selected NHO strain. Owing to restricted space in the laboratory, all the sublines which gave specific tumor types with the same amount of carcinogen received at the same age by the subcutaneous route could not be continued. Consequently, all mice receiving methylcholanthrene at 60 days of life (from the NH strain) are referred to as the NHO subline.

A partial survey of the various types of tumors obtained in approximately 2,000 mice of the NHO strain receiving a subcutaneous injection of 1 mg. of methylcholanthrene dissolved in 0.1 cc. sesame oil at 60 days of life has already been published.[10].

In the first experiment of the present study, dealing with the analysis of a possible sex influence on the induction of tumors, 112 mice (49 females and 63 males) were included. Biweekly observations of the mice after the injection of the carcinogen disclosed the time at which the local tumor at the site of injection in each mouse began to grow. The interval of time from the injection to the growing tumor has been called the latent period. As soon as it was certain that the tumor was growing the animal was put to death. A section of the tumor was fixed in Bouin's fluid and stained with eosin and hematoxylin. There is only one major class of tumor-bearing mice included in the present study that has not already been reported in this series. This class includes those mice in which carcinoma of the lungs developed without the presence of any local or other tumor. There is a considerable number of these mice. The complete analysis of the data bearing on carcinoma of the lungs in NHO mice will be given at some future time. For the present purpose it is perhaps only necessary to state that the latent period for carcinoma of the lungs in those mice which died showing only this internal neoplasm was computed arbitrarily as the age at death minus 30 to 60 days, on the average, depending on the size and the number of tumors in the lungs. The size and the number of tumors both increase with time after the injection of methylcholanthrene, and it is believed, therefore, that this arbitrary estimation of the latent period for this internal neoplasm is near the true value. If there is any discrepancy in the determination of the latent period on adenocarcinoma of the lung, it will not distort the values for the two sexes discriminately at the expense of one of them.

In the second experiment the first 1,000 mice of the same selected subline of the NHO strain are included. Of these, 485 were females and 507 were males. The sex of the remaining 8 was not determined.

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9. Strong, L. C., and Smith, G. M.: Yale J. Biol. & Med. **13**:489, 1941.

10. (*a*) Strong, L. C.: Cancer Research **1**:572, 1941. (*b*) Strong, L. C., and Williams, W. L.: ibid. **1**:886, 1941. (*c*) Strong, L. C.; Collins, V. J., and Durand, E. A.: ibid. **3**:21, 1943. (*d*) Strong.[7]

RESULTS

The rates at which tumors appeared in the mice of the first experiment are recorded in chart 1. The latent periods are given on the base line; the percentages of mice with growing tumors are placed on the vertical. That is, the data for the succeeding ten day periods are cumulative. The data for males are given on the solid line; comparable data for females, on the dash line. By an inspection of the chart it may be seen that there are 12 per cent more males with growing tumors than females, especially during the later part of the experiment as the mice grow older (males, 50 of 63, or 79.3 per cent; females, 33 of 49, or 67.3 per cent). The remaining mice, 13 males and 16 females, died of other causes than induced tumors.

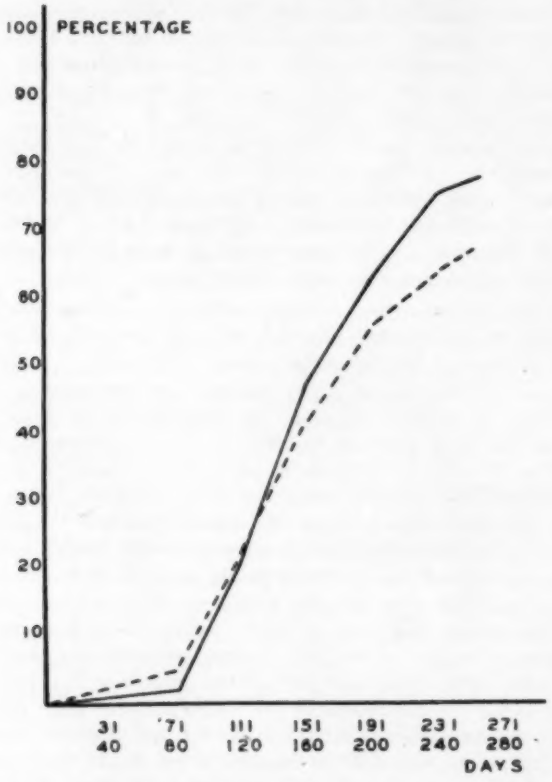

Chart 1 (experiment 1).—The latent period of the induced tumor. The figures for the latent periods are plotted along the base line; the cumulative percentages of incidence, along the vertical line. The data for females are given on the short dash line; the data for males are given on the solid line.

The average induction time for tumors in females was 152.6 days; that for males, 155.3 days.

Data from the second experiment are given graphically in chart 2. An analysis of these data shows that there is no difference in incidence of induced tumors between the sexes; tumors developed in 81.8 per cent of the females and in 79.8 per cent of the males (difference, 2.0 per cent). That is, of the original 485 females in the experiment, 396 presented tumors (of one nature or another). Four of these females are still alive at ages from 550 to 750 days following the injection

of methylcholanthrene. The remaining 85 females died of causes other than tumors. Of the 507 males in the experiment, 405 presented tumors and 7 are still alive at ages between 550 and 750 days following the injection of methylcholanthrene. The remaining 95 died of other causes. If from the total number of females in which tumors developed 22,[10b] the number in which adenocarcinoma of the mammary gland developed, is subtracted, the slight difference between the sexes is reversed (77.1 per cent of females to 79.8 per cent of males). The deletion of this class of tumors is justified since it is restricted to females under spontaneous as well as under methylcholanthrene-induced experimental conditions (sex restricted).

COMMENT

One milligram of methylcholanthrene injected into a mouse is certainly a massive dose. This particular quantity was used in the present investigation for the reason that it was originally intended to produce, if possible, by selective

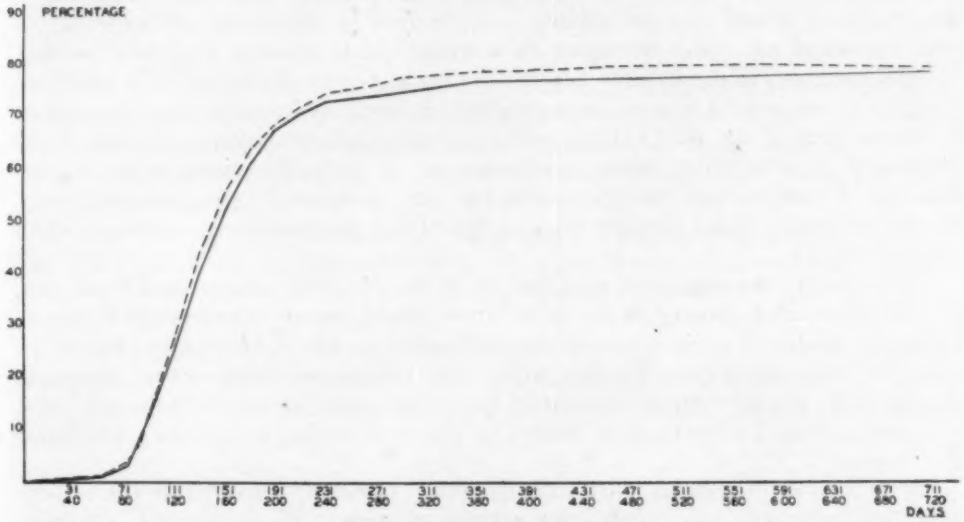

Chart 2 (experiment 2).—The latent period of the induced tumor. The figures for the latent periods are plotted along the base line; the cumulative percentages of incidence, along the vertical line. The data for females are given on the short dash line; the data for males are given on the solid line. There is, therefore, no difference between the sexes in the induction of tumors by subcutaneous injection of 1 mg. of methylcholanthrene dissolved in 0.1 mg. of sesame oil.

inbreeding, following a hybridization experiment, a strain that was entirely resistant to induction of tumors by methylcholanthrene, one of the most potent of all the carcinogens. That is, the criticism that perhaps insufficient amounts of methylcholanthrene were responsible for the nonappearance of tumors was to be avoided. The series of 1,000 mice reported in this paper belonged to the early generations of this selection experiment.

The value of the present investigation on the role of sex in the induction of tumors by a carcinogen may be briefly stated. First, for the final genetic analysis of the problem of the induced tumor all variables must be known and evaluated as completely as possible. Second, the practical application of hormones to the induced tumor, particularly those associated with the phenomenon of sex (which have led to such fundamental conclusions as to the nature of susceptibility to

spontaneous adenocarcinoma of the mammary gland and other genital tissues), should depend to some extent, at least, on the conclusion reached as to whether sex has or has not any influence on the induction of such tumors.

The present data indicate that the sex difference observed in the first experiment on the use of NHO mice (12 per cent in a total of 112 mice) is not a significant one. Consequently, it is probably true that some of the discrepancies in the data reported in the literature and cited on foregoing pages are due, perhaps, to the use of inadequate numbers of experimental animals. The tumors involved in this series, induced by methylcholanthrene, are nonsexual, arising in tissue not identified as of a primary or a secondary sexual nature. The sex hormones, such as estrogen, have, primarily, a very specific effect on genital tissues.

In the present series of 1,000 mice, which were given 1 mg. of methylcholanthrene at 60 days of life, adenocarcinoma of the mammary gland has developed in 22 females.[9b] This tumor group is the only one so far analyzed that shows a difference in sex distribution, the tumor being restricted to the females only. In this case, however, the situation is not the same as it is when adenocarcinoma of the mammary gland may be induced in male mice by estrogens. In order to do this, one must use males belonging to a strain which shows a high susceptibility to similar tumors in the females. In the present experiment the methylcholanthrene was not a measure of genetic susceptibility—it certainly replaced the latter, since in female mice of the NHO strain under normal conditions adenocarcinoma of the mammary gland never develops spontaneously. It is highly probable that a combination of estrogen and methylcholanthrene may occasionally give adenocarcinoma of the mammary gland in male mice of the NHO strain, whereas estrogen alone may not do so.

It is clearly demonstrated that tumors of the NHO strain derived from nongenital tissue and arising in the mice after subcutaneous injection of 1 mg. of methylcholanthrene are not measurably influenced by the difference in physiologic states between the sexes. Further work with minimal amounts of the carcinogen may indicate whether sex is a factor in these cases, also, in the NHO strain, as it has been indicated to be in other strains by the work of Sall and Shear [4] and Shear and Leiter.[5]

In a review, "Estrogens in Carcinogenesis," Gardner [11] arrived at the conclusion that "estrogens may increase the activity of some of these chemicals (carcinogenic), but the changes are apparently slight." He further stated that "the susceptibility of mice of different strains to carcinogenic chemicals does not seem to be more than incidentally associated with the tendency to have mammary tumors." Recently three papers bearing on the effects of estrogens on chemically induced tumors may be of interest. Smith, Wells and D'Amour [12] gave rats injections of methylcholanthrene and estradiol benzoate. They reported that neither castration nor the presence of estradiol benzoate had any effect on the incidence of tumors or the period of latent induction. Segaloff,[13] using CHI mice, reported that relatively large doses of α-estradiol-3-benzoate "had no effect on the incidence, degree of malignancy, or latent period of tumor induction by . . . 20-methylcholanthrene." This negative result was obtained even in hyperestrogenized mice. On the other hand, Paletta and Max,[14] using 40 mice of the Swiss stock, concluded that "the application of α-estradiol benzoate accelerated the transformation of benign tumors into malignant tumors in methylcholanthrene skin carcinogenesis

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13. Segaloff, A.: Cancer Research **2**:794, 1942.
14. Paletta, F. X., and Max, P. F.: J. Nat. Cancer Inst. **2**:577, 1942.

in virgin female mice," and further "that estrogens shortened the induction period for the appearance of carcinomas in the estrogenized mice." Apparently more work is necessary before the final analysis will be forthcoming. Perhaps some of the discrepancies reported in the literature are due to the use of insufficient numbers of experimental animals under controlled conditions.

The negative findings with (1) castration, (2) injection or application of estrogens within reasonable physiologic limits and (3) distribution of tumors in intact animals would certainly indicate that sex as such is not a factor in the induction of tumors by the carcinogens—at least with the relatively large or massive doses of methylcholanthrene and other carcinogens now being used. The work of Sall and Shear [4] and of Shear and Leiter [5] with the use of minute quantities of carcinogens should certainly open up a new field of investigation. But the placing of experimental cancer research on such a high degree of quantitative precision by the use of 0.08 mg. of carcinogen probably necessitates the placing of other variables, such as body weight of animals, age determined in days and the diet which the animals are fed, on a comparable quantitative basis.

SUMMARY

With the use of relatively hybrid mice of the F_3-F_8 generations following an outcross (relatively high biologic variability), a difference of 12 per cent between the sexes in incidence of tumors induced by subcutaneous injection of 1 mg. of methylcholanthrene at 60 days of life (obtained on the use of 112 mice) is not a significant difference.

An analysis of the data obtained on the use of 1,000 animals (485 females and 507 males) demonstrates that in normal mice there is no sex difference in susceptibility to the induction of tumors by subcutaneous injection of 1 mg. of methylcholanthrene dissolved in sesame oil.

A brief survey of the literature bearing on the role of sex hormones, such as estrogen, in relation to tumors induced by carcinogenic chemicals indicates that the process of oncogeny induced by chemicals is probably independent of the activity of such hormones.

THERAPEUTIC EFFECTS OF DISODIUM FORMALDEHYDE SULFOXYLATE DIAMINODIPHENYLSULFONE IN EXPERIMENTAL TUBERCULOSIS

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The publication in 1938 of the report by Rich and Follis [1] on the effects of sulfanilamide on experimental tuberculosis of guinea pigs provided the impetus for the resumption of the search for a chemical agent that could be used successfully in combating clinical tuberculosis of human beings. In the five years that have followed the publication of that report it has been established that (1) a few of the many sulfonamide compounds tried have been capable of exerting a limited but definite deterrent effect on the expected course of the tuberculosis developing on inoculation of guinea pigs [2]; (2) none of the sulfonamide compounds tested in vivo to date have exhibited sufficient tuberculotherapeutic efficacy to engender any optimism concerning the clinical application of such drugs; (3) certain compounds derived from 4,4′-diaminodiphenylsulfone have been shown by several investigators to have a moderate to striking therapeutic effect in vivo against infections established by human tubercle bacilli,[3] and (4) although certain of the compounds containing a sulfone nucleus have been found highly effective in influencing favorably the course of tuberculosis in guinea pigs, most of them unfortunately are objectionably hemotoxic.[4] This fact, while of little consequence in dealing with guinea pigs, has definitely limited the amount of most of the drugs of this type that can be given safely to human beings. It appears in treating human beings that the amount of drug adequate for therapeutic expectations is usually within the toxic range, and in some instances the drug cannot be tolerated over the period necessary for a satisfactory course of treatment.

Evidence at hand is sufficient to justify the statement that experimental tuberculosis of guinea pigs can be combated successfully by any one of several derivatives of 4,4′-diaminodiphenylsulfone. The desirable effects can be obtained even when treatment is delayed for as long as six weeks after infection.[5] Before

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1. Rich, A. R., and Follis, R. H., Jr.: Bull. Johns Hopkins Hosp. **62**:77, 1938.

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3. (*a*) Feldman, W. H.; Hinshaw, H. C., and Moses, H. E.: Am. Rev. Tuberc. **45**:303, 1942. (*b*) Smith, M. I.; Emmart, E. W., and Westfall, B. B.: J. Pharmacol. & Exper. Therap. **74**:163, 1942. (*c*) Feldman, W. H.; Mann, F. C., and Hinshaw, H. C.: Am. Rev. Tuberc. **46**:187, 1942. (*d*) Callomon, F. F. T.: ibid. **47**:97, 1943.

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the maximal effect of compounds of this type can be expected in the treatment of tuberculosis of human beings, derivatives must be supplied that can be administered in adequate doses frequently for a considerable period without marked or serious toxic effects on the patient. It is well to emphasize the dissimilarities between tuberculosis and the more acute infections, which often respond dramatically after a few hours' to a few days' treatment with a sulfonamide compound. The nature of the organism of tuberculosis and of the disease it produces precludes the possibility of such dramatic effects from any form of therapy. For the present the experimental chemotherapeutist should welcome opportunities to test in vivo promising compounds that, while having only a fair to moderate degree of effectiveness against an experimental tuberculous infection, may have a toxicity potential importantly less than that of 4,4'-diaminodiphenylsulfone or any of its known and tested derivatives. A derivative that theoretically appeared worthy of tests in vivo is the compound known as disodium formaldehyde sulfoxylate diaminodiphenylsulfone (fig. 1). This compound was supplied by Dr. George W. Raiziss, director of the Dermatological Research Laboratories (division of the Abbott Laboratories), in Philadelphia.

METHODS

Forty-two adult male guinea pigs were utilized. Each was inoculated subcutaneously with 0.0005 mg. of a human strain of tubercle bacilli known as H37Rv.[6] Forty-two days later

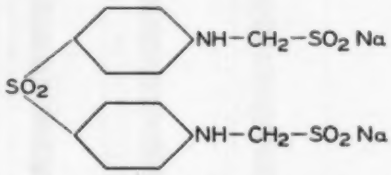

Fig. 1.—Structural formula of disodium formaldehyde sulfoxylate diaminodiphenylsulfone.

all animals were found to be sensitized to mammalian tuberculin administered intracutaneously, and the guinea pigs were divided into two groups. Group 1 consisted of 28 animals and group 2 of 14 animals. The first group was designated the untreated controls. The animals in the second group were to receive treatment.[7] Because of a delay in the availability of the drug, treatment was not started until the forty-sixth day after infection. As in our previous work, we preferred to administer the drug orally with the food. The drug was added to the food to the amount of 0.66 per cent by weight. Usually enough food was prepared to supply the animals for at least two days. Since each animal consumed approximately 50 Gm. of food each twenty-four hours, the intake of the drug per animal during each twenty-four hour period was estimated to be 325 to 350 mg.

The experiment was terminated two hundred and twenty-eight days after the animals had been inoculated with tubercle bacilli. When the experiment ended, the animals in the treated group had received the drug daily for the last one hundred and eighty-two days of the experiment.

At the time of necropsy, composite suspensions were made of portions of the livers and the spleens of 8 of the treated animals. After preliminary treatment with 5 per cent solution of oxalic acid, each suspension was used to inoculate eight slants of egg yolk–agar medium. The slants were incubated at 37.5 C. for sixty days.

6. Detailed information as to the origin and the characteristics of this strain of tubercle bacilli, and data concerning the practice followed in feeding the guinea pigs, will be found in a report [3a] published previously.

7. The untreated animals in this experiment also served as controls for testing concurrently in vivo several other compounds.

RESULTS

Survival Time.—When the experiment was terminated, 20 (71.4 per cent) of the animals in the untreated control group had died, while only 2 (14 per cent) of those that had been treated were dead. The first of the treated animals died one hundred and nineteen days after infection, while the other which died did so one hundred and thirty-three days after inoculation. The cause of the death of the first animal that died was not apparent. There was present in the second guinea pig that died sufficient tuberculosis to account for death.

Relative Amounts of Tuberculosis in the Untreated and Treated Groups of Animals.—The tissues preserved for microscopic examination included in every

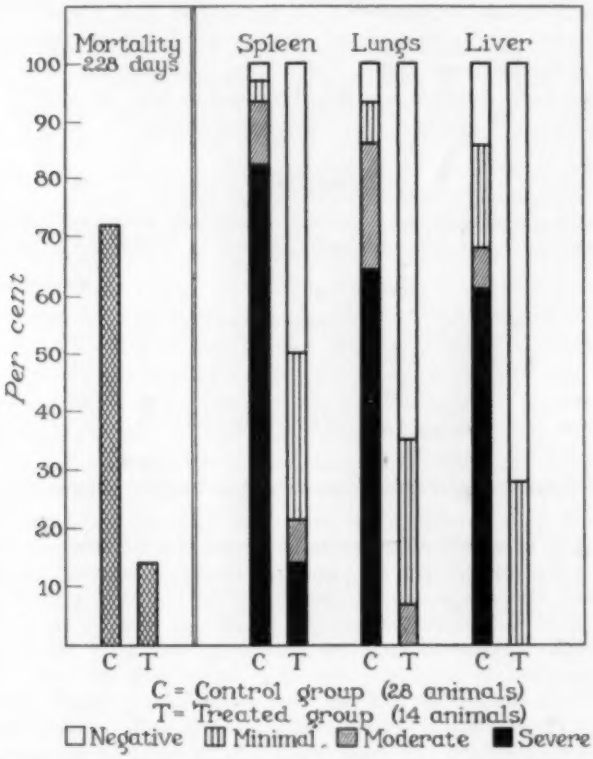

Fig. 2.—Mortality rates in treated and untreated groups of guinea pigs and the comparative severity of the tuberculous involvement in the organs of predilection.

instance the spleen, the liver, the lungs, the tracheobronchial lymph nodes, the subcutis at the site of injection and the lymph nodes of the axillary regions.

Grossly and microscopically there was significantly less tuberculosis among the animals that had been treated than among those that had not been treated. Furthermore, the two groups were markedly dissimilar in the character of the tuberculosis (fig. 2).

The average severity of tuberculosis in the treated and in the untreated group of guinea pigs is expressed numerically in table 1.[8] In table 1 will be

8. A description of the scheme followed in recording numerically tuberculous lesions of experimentally infected guinea pigs is given in another communication.

found also data pertaining to the results obtained in a concurrent experiment with 4,4′-diaminodiphenylsulfone. It is clearly evident that the amount of tuberculosis observed in each of the treated groups of guinea pigs was impressively less than the amount recorded for the untreated controls, the respective average indexes being 8.4, 12.6 and 84.1. The data indicate that 4,4′diaminodiphenylsulfone was more effective in controlling or arresting the course of the infection in the spleen than was disodium formaldehyde sulfoxylate diaminodiphenylsulfone. The relative degrees of effectiveness of the two compounds in the other anatomic situations were comparable. It is evident that each of the drugs was fairly successful in exerting a favorable influence on a disease process that was capable in the untreated animals of widespread destructive and lethal effects.

Histologic Evidence of Chemotherapeutic Effects.—The pathologic changes that characterized the disease in the untreated controls were typically those which one would expect in guinea pigs inoculated with virulent tubercle bacilli of the human type which have had an opportunity to exert a considerable measure of their potential pathogenicity. While it is true that approximately 29 per cent of the untreated animals were still living when the experiment was

TABLE 1.—*Average Severity of Tuberculosis in Different Organs, Expressed Numerically* *

Group	Spleen (Max. 35)	Lungs and Contiguous Lymph Nodes (Max. 30)	Livers (Max. 25)	Site of Inoculation (Max. 10)	Average Index of Infection (Max. 100)
Control (28 animals)	31.3	24.7	18.4	9.7	84.1
4,4′-diaminodiphenylsulfone (13 animals)	1.4	2.8	0.23	4.0	8.4
Disodium formaldehyde sulfoxylate diaminodiphenylsulfone (14 animals)	7.3	1.7	0.57	3.0	12.6

* The duration of the experiment was two hundred and twenty-eight days. Treatment with disodium formaldehyde sulfoxylate diaminodiphenylsulfone was started on the forty-sixth day after infection. Treatment with 4,4′-diaminodiphenylsulfone was started on the forty-second day after infection. Treatment was continuous and varied from a minimum of thirty days to a maximum of one hundred and eighty-six days, depending on the duration of life of the respective animals in the treated group.

terminated, the amount of tuberculous infection in the 8 untreated animals that were killed was quite comparable to that recorded previously for the untreated animals that had died. Microscopically, the lesions in the untreated controls were typically those of unrestrained, progressively destructive tuberculosis, which in the majority of instances involved the bulk of the spleen, lungs and liver. In most animals in the untreated group, an open ulcerative lesion marked the site of inoculation, and the lymph nodes in the contiguous regions showed marked adenopathy and caseation necrosis.

Among the 14 spleens from the animals treated with disodium formaldehyde sulfoxylate diaminodiphenylsulfone were 3 in which there were moderate to extensive lesions of progressive tuberculosis and 4 in which the lesions appeared to be nonprogressive or arrested. In 7 spleens no demonstrable signs of tuberculosis were found grossly or microscopically. From a histologic point of view the 4 spleens containing nonprogressive or arrested lesions were of much interest. In 3 of these, the lesions were few, and the bulk of the splenic tissue was normal in appearance. The lesions of tuberculosis in these spleens when compared with those in the spleens from the animals that had not been treated were significantly dissimilar. The majority consisted of discretely situated nodules of epithelioid cells in which fibroblastic transitional changes were readily discernible. Necrosis or other signs of destructive progression such as characterized the lesions in the untreated animals were absent (fig. 3 *a* and *b*). Although, as mentioned pre-

viously, the treatment failed to influence favorably the course of the disease in 3 spleens, the influence of the drug in the remaining 11 spleens is worthy of emphasis.

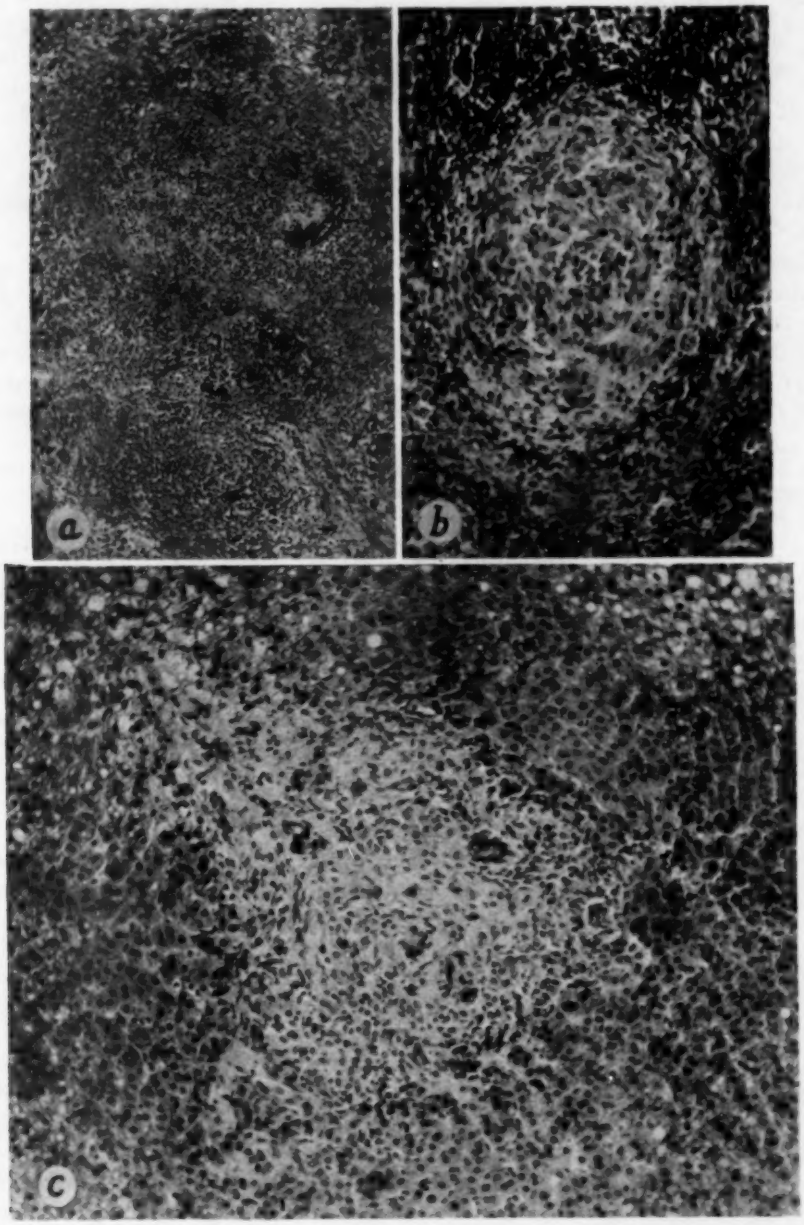

Fig. 3.—(*a*) Spleen from an untreated control, showing severe destructive changes (× 60). (*b*) Spleen from a guinea pig treated one hundred and eighty-two days; a single discrete "hard tubercle" is seen (× 130). Both animals had been infected for 228 days. (*a*) and (*b*) show the characteristic dissimilarity of the morbid processes. (*c*) Liver of a guinea pig which died one hundred and thirty-three days after infection and which had been treated for eighty-seven days. A nonprogressive tuberculous lesion is seen, consisting of epithelioid cells and transitional fibroblastic forms (× 130).

The tuberculous changes in the livers of the animals that were treated were few. In only 4 of the 14 livers were lesions demonstrable. In 3 the lesions were discernible grossly, while in 1 the lesions were found only microscopically. In none of the 4 livers were the lesions numerous, and in 1 very few foci were found. Morphologically the lesions were essentially similar. They consisted of small, compact nodules composed of epithelioid cells. Fibroblastic changes were evident, especially in the more peripheral portions of the lesions (fig. 3 *c*). In some instances, peripheral encapsulation was beginning, and variable numbers of lymphocytes were present. In none of the involved livers did the disease appear aggressive—this in spite of the fact that in 3 instances the lesions represented an infection of two hundred and twenty-eight days' duration.

As was true in the livers, relatively little tuberculosis was found in the lungs of the animals that were treated with disodium formaldehyde sulfoxylate diaminodiphenylsulfone. Histologic evidence of tuberculosis was noted in the parenchymal tissues of the lungs of 4 animals, while in 9 the lungs proper were devoid of demonstrable lesions. However, tuberculous changes occurred in the tracheobronchial lymph nodes of 6 animals other than those that had parenchymal lesions. The possibility or likelihood of the tracheobronchial lesions representing residual lesions from a focus or foci originally present in the lungs seems a reasonable hypothesis.

Microscopically, the lungs of only 1 animal contained lesions that could be considered possibly progressive. In this instance, although both lungs were affected, the involvement was graded as slight. The signs of progression were not impressive, since the regions of involvement were localized and the greater proportion of the pulmonary tissue was not affected. Incidentally, lesions of tuberculosis were not found in the liver or in the spleen of this animal. The lesions in the lungs of the other 3 animals were presumably tuberculous, although they were not characteristic. In 1 animal the only lesion found occurred in the peribronchial tissues and consisted of a small fibrotic nodule that could conceivably have represented a healed tubercle (fig. 4 *a* and *b*). In another animal the only lesion found in the lungs was a small plaque of what looked like bone or fibro-osteoid tissue (fig. 4 *c*). This may or may not have been related to a previous tuberculous state, although this possibility is worthy of consideration.[9] The pulmonary lesions in the last of the 4 animals that had pulmonary involvement were few and consisted of small foci of epithelioid cells and histiocytes.

The tuberculous lesions of the tracheobronchial lymph nodes varied in character from those which occupied or had replaced most of the lymphoid tissue to discrete foci with much of the lymphoid tissue intact. In all instances there was marked fibroblastic transition of the epithelioid elements. One of the nodes contained a large lesion in which there was extensive central necrosis.

That the therapeutic effect of the drug had been exerted favorably against the infection at the site of inoculation and in the contiguous lymph nodes of the axillary region was indicated by the fact that residual lesions were found at the sites of inoculation in only 5, and in the axillary lymph nodes in 9, of the 14 treated guinea pigs. For comparison it may be noted that among the 28 untreated or control animals, residual lesions were found at the sites of inoculation in 26, while the disease was demonstrable in the axillary lymph nodes in each of the 28.

9. Similar lesions have been observed occasionally in the lungs of tuberculous guinea pigs treated with sodium p,p'-diaminodiphenylsulfone-N,N'-didextrose sulfonate (promin). Lesions of this character have not been encountered in any of the large number of untreated animals observed.

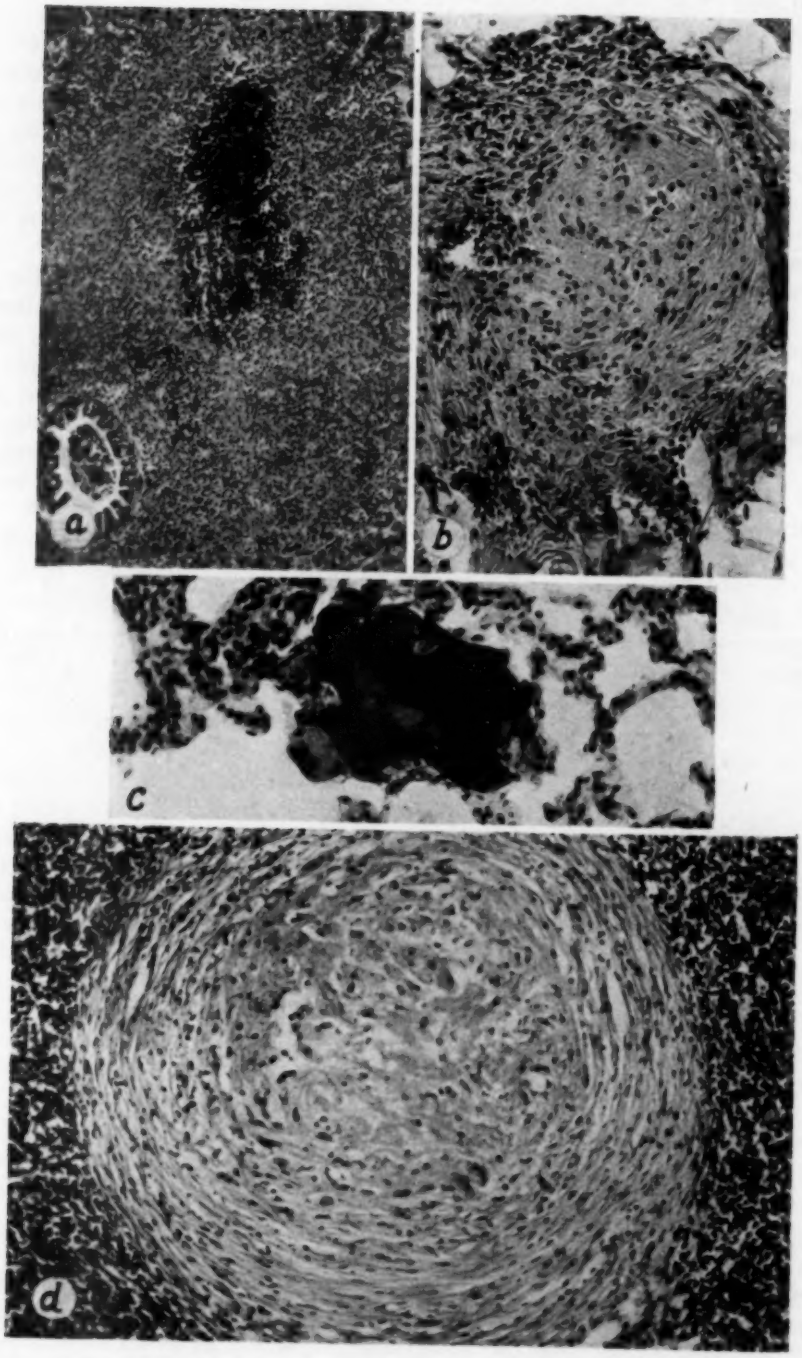

Figure 4

(See legend on opposite page)

Microscopically, 3 of the treated animals were considered to have slight but progressive disease at the site of inoculation or in the contiguous lymph nodes, while the others either showed no lesions or lesions in an arrested state (fig. 4 *d*). A few foci of calcification were noted.

Attempt to Recover Tubercle Bacilli from Tissues of Treated Animals.—With regard to the composite suspensions made from portions of the livers and the spleens of 8 of the treated animals, the results were negative in all instances except 1. The positive result consisted of a single colony of acid-fact bacilli on one of the eight slants inoculated with material from 1 of the 3 guinea pigs that had morphologically progressive tuberculosis of the spleen.

Sensitivity to Tuberculin.—As mentioned previously, all of the guinea pigs were found to be sensitized to tuberculin at the time treatment began. When the experiment was terminated, one hundred and eighty-two days later, the animals were again tested with tuberculin. Of the 12 treated guinea pigs subjected to the second test, all but 1 gave a definitely positive reaction. In the 1 animal the reaction was recorded as "indefinite." [10]

Toxicity.—The study of disodium formaldehyde sulfoxylate diaminodiphenylsulfone did not include a comprehensive investigation of its toxicity for guinea pigs. The information we have concerning its toxic effects was assembled from the routine procedures followed in the course of the experiment to determine its tuberculotherapeutic efficacy. The information on hand concerns the tolerated dose given orally for one hundred and eighty-two days, the effects on the kidneys, the spleen, the liver and the lungs as determined morphologically, and the effects on the blood.

It was found that guinea pigs did not tolerate satisfactorily a dose of 1 per cent of the drug in the food. After a few days on this dose the animals showed definite inappetence, with reduction of the intake of food from an average of 50 Gm. daily to amounts as low as 30 Gm. daily. Consequently, the amount of the drug added to the food was reduced to 0.66 per cent by weight. This amount was tolerated satisfactorily. The animals ate well and did not exhibit any objective signs of toxicity.

Microscopically, no tissue changes that suggested a toxic effect on the part of the drug were detected in the parenchymatous organs.

At the termination of the experiment our colleague Dr. George M. Higgins obtained blood by cardiac puncture from animals in the control group, the group that had been treated with 4,4′-diaminodiphenylsulfone and the group that had been treated with disodium formaldehyde sulfoxylate diaminodiphenylsulfone. From

10. At necropsy the only demonstrable tuberculosis in this animal was a nonprogressive focus in one of the axillary lymph nodes.

EXPLANATION OF FIGURE 4

(*a*) Region of tuberculous consolidation with necrosis in a lung of an untreated animal dying one hundred and ninety-four days after infection (× 60).

(*b*) Small fibrotic or healed nodule in a lung of an animal treated for one hundred and eighty-two days (× 130).

(*c*) Possible bone or fibro-osteoid tissue in a lung of a guinea pig treated for one hundred and eighty-two days. The animal was killed two hundred and twenty-eight days after infection. No lesions were found in the liver or the spleen (× 180).

(*d*) Tuberculous nodule in an axillary lymph node of a guinea pig treated for one hundred and eighty-two days. The animal was killed two hundred and twenty-eight days after infection. Only a few epithelioid cells are discernible. The striking fibroblastic changes are indicative of nonprogression or even healing (× 130).

each specimen the following items were determined: (1) number of erythrocytes per cubic millimeter, (2) volume of erythrocytes in cubic microns, (3) grams of hemoglobin per hundred cubic centimeters of blood and (4) percentage of erythrocytes recognized as reticulocytes. The results of this portion of the study are summarized in table 2.

It seems clear from the data in table 2 that the toxic effects exerted on the erythrocytes were more marked in animals which received 4,4′-diaminodiphenylsulfone than in those which received disodium formaldehyde sulfoxylate diaminodiphenylsulfone. The average erythrocyte level in animals receiving the latter drug was significantly lower than the level found in the control animals; yet it was significantly higher than that observed in animals which received 4,4′-diaminodiphenylsulfone, the difference (850,000 ± 160,000 cells) being more than five times its own standard error.

The same conclusion is indicated when the data on volume of erythrocytes and those on percentage of reticulocytes are similarly contrasted. In each category, the values are significantly improved in animals given disodium formaldehyde sulfoxylate diaminodiphenylsulfone as compared with those of animals which received 4,4′-diaminodiphenylsulfone. The values for hemoglobin, on the other hand, are of the same statistical order in the two groups of experimental animals.

TABLE 2.—*Comparative Data Pertaining to the Effects on the Blood of Guinea Pigs of 4,4′-Diaminodiphenylsulfone and Disodium Formaldehyde Sulfoxylate Diaminodiphenylsulfone*

Group	Erythrocytes per Cubic Millimeter	Erythrocyte Volume, Cubic Microns	Hemoglobin, Gm. per 100 Cc. of Blood	Reticulocytes, per Cent of Erythrocytes
Control (8 animals)	5,350,000 ± 50,000*	84.9 ± 0.90	13.3 ± 0.15	1.3 ± 0.17
4,4′-diaminodiphenylsulfone (6 animals)	3,630,000 ± 140,000	123.1 ± 2.3	11.2 ± 0.5	10.3 ± 0.8
Disodium formaldehyde sulfoxylate diaminodiphenylsulfone (6 animals)	4,480,000 ± 80,000	90.5 ± 1.8	12.1 ± 0.4	7.1 ± 0.3

* Probable error of the mean.

These average values are both significantly lower than those of the controls but do not differ significantly from each other.

As far as the erythrocytes are concerned, the conclusion seems indicated from observations on the total number of erythrocytes and their volume, as well as on the percentage of reticulocytes (which indicates destruction of cells and subsequent regeneration), that disodium formaldehyde sulfoxylate diaminodiphenylsulfone is far less toxic than 4,4′diaminodiphenylsulfone.

COMMENT

The results of our study of disodium formaldehyde sulfoxylate diaminodiphenylsulfone agree in general with those of Callomon,[3d] who likewise studied the effects of this drug in vivo. Although our methods of procedure were different in several important respects from those followed by Callomon, our results indicate, as did his, that this compound is capable of exerting a considerable deterrent effect on the course of experimental tuberculosis in guinea pigs. The favorable effect of this drug is indicated by the fact that the mortality rate of the treated animals was approximately a fifth of that of the untreated controls and by the fact that the amount of disease in the treated animals was strikingly less than occurred in those animals that were not treated. Of much importance also was the dissimilarity of the two groups of animals as regards the character of

the disease. Whereas the infective process in the untreated animals was characterized by widespread dissemination and by progressive and destructive behavior, that in most of the animals that had been treated either was not demonstrable in the parenchymatous organs or was minimal in extent and in an arrested or nonprogressive state. These facts constitute evidence for believing that disodium formaldehyde sulfoxylate diaminodiphenylsulfone has a rather high tuberculotherapeutic efficacy so far as experimental tuberculosis of the guinea pig is concerned. Its toxicity for guinea pigs in the dose used was only moderate and definitely less than that of the parent substance from which it is derived. Likewise it was less effective in combating tuberculosis under conditions comparable to those that obtained in testing the parent substance (4,4′-diaminodiphenylsulfone).

SUMMARY AND CONCLUSIONS

Beginning forty-six days after subcutaneous inoculation with 0.0005 mg. of human tubercle bacilli (strain H37Rv), 14 guinea pigs were treated daily with a derivative of 4,4′-diaminodiphenylsulfone known as disodium formaldehyde sulfoxylate diaminodiphenylsulfone. The drug was administered orally in the food, which contained 0.66 per cent by weight. The average daily intake of the drug was estimated at 325 to 350 mg. for each animal. A group of 28 animals that were not treated served as controls. The experiment was terminated two hundred and twenty-eight days after the animals had been infected. Approximately 71 per cent of the untreated animals died, compared with approximately 14 per cent of the animals in the treated group. Markedly less tuberculosis was found in the group that was treated than in the controls, and the character of the disease in the treated animals provided morphologic evidence that the drug exerted a considerable deterrent effect.

The following conclusions are drawn: 1. Disodium formaldehyde sulfoxylate diaminodiphenylsulfone represents another compound containing a sulfone nucleus and capable of an effective therapeutic result in experimental tuberculosis. 2. This drug is apparently less toxic for guinea pigs than 4,4′-diaminodiphenylsulfone, from which it is derived. Conversely, it is also somewhat less effective than the parent substance in its capacity to deter tuberculous infections in guinea pigs.

Case Reports

FIBROSARCOMA OF THE SKULL IN PAGET'S DISEASE

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The association of osteogenic sarcoma of the long bones and osteitis deformans (Paget's disease) in the same person is not infrequent, whereas that of sarcoma of the skull and Paget's disease is quite rare. Paget [1] in his original paper noted that bone tumor had been present in 3 of his 7 cases. Schmorl [2] in reviewing the anatomic findings in 4,600 spinal columns encountered Paget's disease in 138, an incidence of 3 per cent in persons past 40 years of age. The skull was involved in 39 cases, being fourth in frequency of involvement. Since the concomitant finding of sarcoma of the skull is so rare, I have been prompted to report an additional case.

Sarcoma of the skull in Paget's disease has been reported by Packard, Steele and Kirkbridge [3] (1 case), Bird [4] (2 cases), Locke [5] (1 case), Jaffe [6] (1 case), Davie and Cooke [7] (1 case)) and Albee [8] (1 case). The Registry of Bone Tumors

TABLE 1.—*Incidence of Bone Sarcoma in Paget's Disease*

Author	Cases of Paget's Disease	Cases with Sarcoma of the Skull	Cases with Sarcoma in Other Bones
Packard, Steele and Kirkbridge [3]	66	1	7
Bird [4]	64	2	7
Locke [5]	65	1	3
Albee [8]	1	1	1
Davie and Cooke [7]	2	1	2
Jaffe [6]	2	1	2
Kirshbaum	8	1	0
Total	208	8	22

Note.—The Registry of Bone Tumors of the American College of Surgeons has recorded sarcoma of the skull in 2 of 28 cases of Paget's disease.

of the American College of Surgeons [9] has recorded 2 cases. With the case to be reported, sarcoma of the skull has been encountered in 8 of 208 cases of Paget's disease, an incidence of 3.8 per cent. (The cases of the Registry of Bone Sarcoma have not been included.) In this same series there were 22 cases in which sarcoma was also present in other bones, a total incidence of 14.4 per cent, which is quite consistent with the figures usually mentioned in individual reports (table 1).

In a series of 13,000 consecutive necropsies at the Cook County Hospital in Chicago from 1929 to May 1940, inclusive, Paget's disease was encountered eight times and was associated with fibrosarcoma of the skull once.

1. Paget, J.: Tr. M. Clin. Soc. **60**:37, 1877; M. Classics **1**:29, 1936.
2. Schmorl, G.: Virchows Arch. f. path. Anat. **283**:694, 1932.
3. Packard, F. A.; Steele, D. J., and Kirkbridge, T. S.: Am. J. M. Sc. **122**:252, 1901.
4. Bird, C. E.: Arch. Surg. **14**:1187, 1927.
5. Locke, E. R, cited by Bird.[4]
6. Jaffe, H. L.: Arch. Path. **15**:83, 1933.
7. Davie, T. B., and Cooke, W. E.: Brit. J. Surg. **25**:299, 1937.
8. Albee, F. H.: J. A. M. A. **107**:1693, 1936.
9. Crowell, B. C., Registrar of the Registry of Bone Sarcoma: Personal communication to the author.

REPORT OF A CASE

A 78 year old white woman was admitted to the Cook County Hospital July 1, 1936 in a comatose state. It was learned from the family that she had had a mass over the left side of the head for two years and that she had had a stroke previous to the present one, which affected her mental condition. The day before admission to the hospital she suddenly became unconscious and her right arm was found to be paralyzed.

The patient was in a serious condition. She was unable to speak, and there was ptosis of the right eye as well as flaccid paralysis of the right arm. Over the left parietal region of the skull a soft, pulsating mass was felt under the scalp, about the size of an egg. It appeared to pulsate with the temporal artery. The lower lobes of both lungs were dull, and no breath sounds were heard. The heart tones were clear and the rate regular. The abdomen showed no abnormality. In view of the patient's poor condition, surgical exploration was not deemed advisable.

Roentgenograms of the skull disclosed that the bones of the calvarium were about four times the normal thickness. These bones were very spongy and blotchy in appearance, with

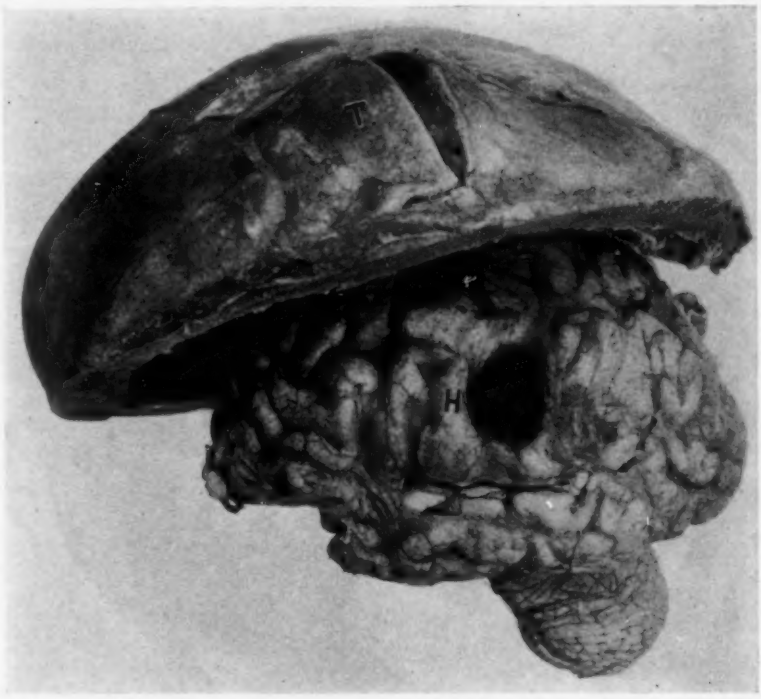

Fig. 1.—Photograph showing the tumor in the skull (*T*) and hemorrhagic softening in the left parietal lobe of the brain (*H*).

loss of the continuity of outline of the inner and the outer tables. There were several circumscribed oval areas of increased density. The left parietal bone was extensively involved, and the sphenoid bone was also unusually dense. The left parietal bone showed, near the junction of the occipital bone, an area of marked absorption of the inner table, measuring about three-quarters of an inch (about 2 cm.) in its largest diameter, which was directed anteroposteriorly.

The Kahn test of the blood was negative. The first examination of the urine gave negative results but a subsequent examination disclosed much sugar.

The patient's condition rapidly became worse; the temperature gradually rose to 107.4 F.; bronchopneumonia developed, and death occurred four days after admission.

Necropsy.—The body was that of a poorly nourished, emaciated senile white woman. The skull appeared prominent, and in the left parietal region, beneath the scalp, there was an elevated firm mass, 7 cm. in diameter. The calvarium when removed measured 19.5 by 17.5 cm., weighed 865 Gm. and varied in thickness from 12 to 30 mm. The outer table, especially

the occipital portion, was brittle and broke easily on slight manipulation, while the inner table was firm. In the left parietal region there was a circumscribed oval mass, 6 by 4.5 cm., which projected 1.5 cm. above the surface. It was firm, dark purple-tan and mottled with purple-red areas. It extended through the entire thickness of the skull, and on the inner

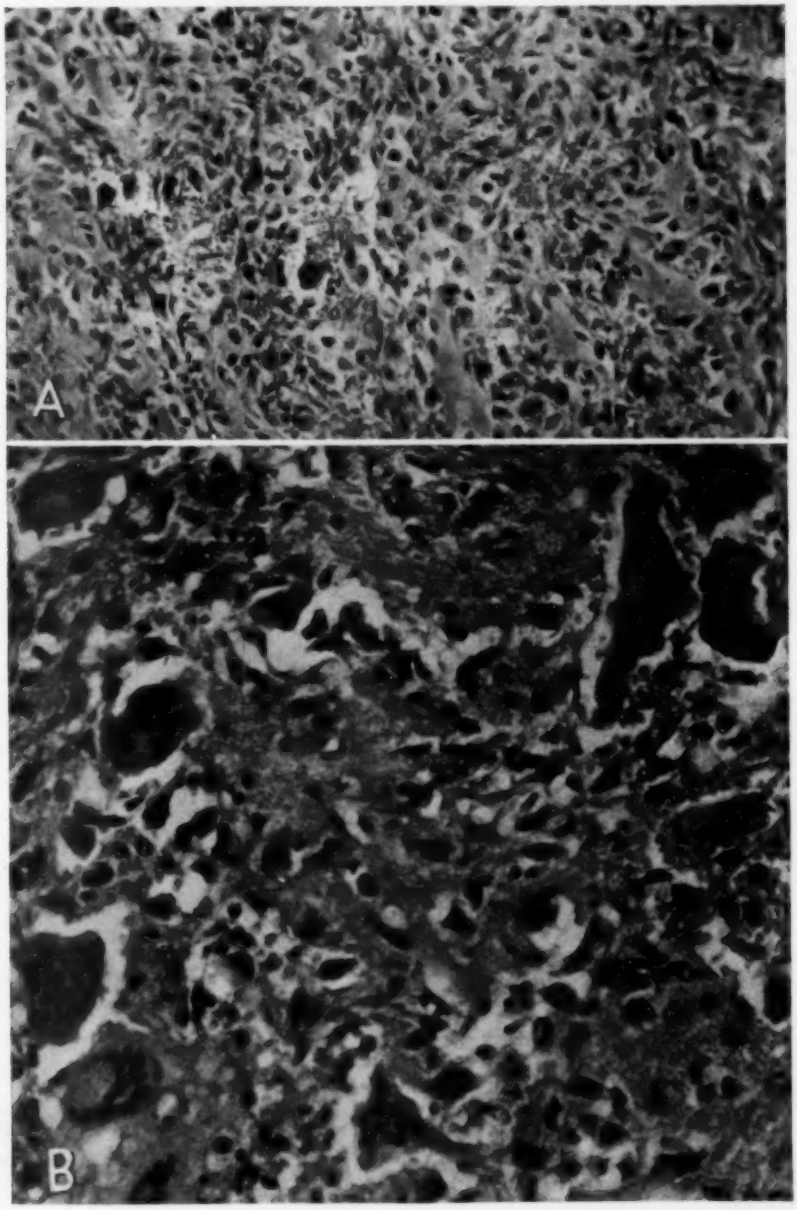

Fig. 2.—Photomicrographs of sarcoma of the skull: *A*, low power. Note the spindle cell character. *B*, high power. Note the similarity to giant cell tumor.

aspect of the skull it measured 3 by 2.5 cm. and bulged into the brain substance for 18 mm. Here it was discolored deep purple-red (fig. 1). The surrounding dura was firmly adherent to the inner table and was reddish brown from congestion.

The brain was much smaller than normal, weighing 890 Gm. It was moderately firm, and the leptomeninges were thickened. The convolutions were moderately flattened. In the

region of the left parietal lobe, just beneath the tumor mass described, there was an oval depression, 25 by 15 by 16 mm. It was dark purplish red, and the vessels were dilated and congested. The right parietal lobe presented a shallow depression, 5 by 3 cm. (fig. 1). The cerebellar hemispheres were markedly compressed by the thickened skull, and on the posterior surface were shallow grooves. The cerebral arteries at the base of the brain were thick walled, and the intima was studded with hyaline and calcific plaques.

The long bones of the lower extremities were short and thick, and presented lateral bowing. The iliac bones and the symphysis pubis were also thickened and appeared prominent.

The other findings were bronchopneumonia in both lower lobes, severe fatty changes in the liver, severe coronary sclerosis, nodose colloid goiter and lipomatosis of the pancreas.

Microscopic Examination.—The tumor of the left parietal bone consisted of large oval to spindle-shaped cells with irregular hyperchromatic nuclei. Many of the nuclei were in the stage of mitotic division. In places the cells were separated by a pale pink-staining homogeneous ground substance resembling osteoid tissue (fig. 2). Scattered about were many free large multinucleated giant cells of the osteoclast type, so that the picture strongly resembled that of a giant cell tumor. In the areas where the giant cells were particularly numerous there were wide blood spaces, frequently containing recent thrombi. The tumor contained in places extravasations of blood and foci of large mononuclear cells and polymorphonuclear leukocytes. Bands of hyaline connective tissue often separated groups of tumor cells. At the periphery of the tumor were spicules of necrotic bone and many free osteoclasts. The inner aspect of the tumor, which formed a knoblike projection and bulged into the parietal lobe of the brain, was chiefly composed of extravasations of blood mixed with tumor cells and multinucleated giant cells containing from 3 to 28 nuclei.

Sections taken from the frontal bone of the skull showed no separation of the internal and external tables but a uniform composition of variously shaped short and thick bony trabeculae surrounded by much loosened connective tissue. The bony trabeculae contained numerous widened cement lines, which subdivided the bone into innumerable small and variously shaped pieces, giving the bone a mosaic pattern.

The bony trabeculae for the most part were lined by numerous fairly large osteoblasts and were surrounded by a layer of osteoid tissue in which were embedded single osteoclasts, giant cells and many fibroblasts. The widened marrow spaces, which showed marked fibrosis, were vascular and infiltrated with small accumulations of lymphocytes and plasma cells and contained recent extravasations of blood. In places, in small excavations of the bone, osteoclasts were seen to be present.

The occipital portion of the skull disclosed a thin fibrosed outer plate, which was separated from the inner vascular table. The inner two thirds of the bone was composed of short trabeculae of bone widely separated by a fine fibrillar reticulum containing numerous dilated and congested capillaries. In places the marrow was fatty, with extravasations of red blood cells and foci of myelopoiesis.

Sections taken from the hypophysis and one of the parathyroid glands failed to reveal any unusual changes.

Anatomic Diagnosis.—The conditions diagnosed were: sclerosing fibrosarcoma of the left parietal bone; osteitis deformans (Paget's disease) of the skull, the pelvic bones and both femurs; hemorrhagic encephalomalacia of the left parietal lobe; bronchopneumonia; lipomatosis of the pancreas; marked coronary sclerosis and moderate sclerosis of the basilar cerebral arteries; nodose colloid goiter; brown atrophy of the myocardium.

COMMENT

In all the 8 cases of Paget's disease studied, the skull showed the typical osseous changes; in 5 cases other bones were also involved. The average age of the patients was 67.5 years; the youngest was 48 years, while the oldest was 85 years. Both sexes were equally affected—4 males and 4 females. Five of the patients were white persons and 3 were Negroes. The primary cause of death was unrelated to Paget's disease in 7 of the cases, while in 1 the sarcoma of the skull predisposed to encephalomalacia and terminal bronchopneumonia (table 2).

The cause of osteitis deformans (Paget's disease) and the cause of osteogenic sarcoma arising in this disease are unknown, but such factors as trauma, fracture and chronic inflammation have been suggested. Hypoparathyroidism has been mentioned as the cause of Paget's disease, but careful examination of the parathyroid glands has given negative results thus far. The finding of serum phosphatase values as much as twenty times normal values has been recorded in cases

showing active formation of bone (Kay [10]), but this rise in phosphatase has not been specific for Paget's disease, since similar high values have been noted in osteitis fibrosa cystica and in rickets. The question of trauma as an etiologic factor for sarcomatous transformation in cases of Paget's disease is discussed by Grizaud,[11] who observed a fracture of the humerus preceding the formation of a tumor diagnosed as osteoblastic sarcoma in a patient known to have had Paget's disease for fifteen years.

It has been a moot question whether osteitis deformans predisposes to sarcomatous transformation in affected bones. Is Paget's disease a presarcomatous condition? The following pathologic processes may be considered as factors in the cancerous transformation of the bones affected by Paget's disease: (1) continuous metaplasia of connective tissue to new bone formation; (2) continuous resorption of the old bone by osteoclastic giant cells, and (3) active substitution of trabeculae of bone by vascular connective tissue.

It is this potential proliferative capacity of the tissues which may be the initial stimulus for tumor formation, or perhaps it is the abnormal exaggeration of these pathologic processes which is apt to induce a sarcomatous neoplasm in the affected

TABLE 2.—*Summary of Data on Eight Cases of Paget's Disease in Which Sarcoma of the Skull Developed*

Case	Age of Patient	Race	Sex	Bones Involved	Diagnosed Clinically	Anatomic Cause of Death
1	67	Negro	M	Skull	No	Tuberculous meningitis
2	64	White	M	Skull, clavicle, tibia, femur	Yes	Hypertensive heart disease
3	77	White	F	Skull	No	Encephalomalacia
4	48	White	M	Skull, spinal column, right tibia	Yes	Suppurative pachymeningitis, hypertensive heart disease
5	58	Negro	F	Long bones, skull	Yes	Coronary disease, hypertensive heart disease
6	78	White	F	Skull, femur, pelvis	Yes	Bronchopneumonia, encephalomalacia
7	63	White	M	Skull, pelvis, spinal column, femur, left tibia	Yes	Bronchopneumonia, syphilitic aortitis
8	85	Negro	F	Skull	No	Peptic ulcer, cerebral sclerosis

bones. On the other hand sarcoma is just as apt to develop in patients past middle life without Paget's disease. In the same series of necropsies bone sarcoma was observed in 8 patients past 50 years of life. Thus one could argue that Paget's disease does not predispose to cancerous transformation in an affected bone but is merely an incidental finding. However, one cannot overlook the high incidence of sarcoma in bones affected by Paget's disease, as mentioned by Packard, Steele and Kirkbridge [3] (12.1 per cent), Bird [4] (14 per cent), Locke [5] (6.1 per cent), Crowell [9] (14 per cent) and Kirshbaum (12.5 per cent).

In the cases of bone sarcoma described there was often an antecedent history of Paget's disease for as long as fifteen years (Jeanneney and Cretin,[12] Breslich [13] and others); the relationship of the two conditions may therefore be more than mere coincidence.

Sarcoma in the bones of patients with Paget's disease may have multicentric foci of origin, as observed by Jaffe [6] and by Parenti and Lüdeke.[14] Jaffe's patient showed multiple sarcomatous foci in the skull and other regions of the skeleton.

10. Kay, H. D.: J. Biol. Chem. **89**:249, 1930.
11. Grizaud, H.: Presse méd. **44**:1018, 1936.
12. Jeanneney, G., and Cretin, A.: Ann. d'anat. path. **13**:664, 1936.
13. Breslich, P. J.: Arch. Surg. **23**:918, 1931.
14. Parenti, G. C., and Lüdeke, H.: Virchows Arch. f. path. Anat. **296**:200, 1935.

SUMMARY

A case of giant cell fibrosarcoma of the skull associated with Paget's disease in a 78 year old white woman is described. The tumor had extended into the left parietal lobe, producing hemorrhagic encephalomalacia.

Paget's disease was encountered eight times in 13,000 necropsies; it was associated with sarcoma of the skull once.

Every case of sarcoma of the skull should be investigated for the coexistence of Paget's disease.

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DIFFUSE GUMMATOUS MYOCARDITIS

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The case of diffuse gummatous myocarditis in congenital syphilis presented here is believed to be sufficiently interesting and unusual to warrant recording.

A Negro girl a few days more than 2 months old, born of a syphilitic mother, was seen in the outpatient department with a cough and a mild fever of two weeks' duration. Diminished breath sounds and inconstant rales were heard over the left lung. A roentgenogram showed definite infiltration in the upper lobe of the right lung and enlargement of the heart to both the right and the left. The leukocyte count was 15,400. Sulfathiazole (2-[para-aminobenzenesulfonamido]-thiazole) was prescribed.

Ten days later the child was admitted to the hospital acutely ill. The temperature was 103.2 F., the pulse rate 102 and the respiratory rate 36. The respirations were labored, with retraction of the ribs bilaterally. There were scattered rales in the bases of the lungs with diminished breath sounds over the lower lobe of the left lung. The liver was palpable 2 cm. below the right costal margin.

The leukocyte count was 18,000. The Wassermann and Kline reactions of the blood were positive. The Wassermann reaction of the spinal fluid was positive, but the Kline test gave a doubtful result.

The baby was placed in a croup tent and was given transfusions of whole blood and plasma. Her respirations grew more labored, and she became cyanotic and died six and a half hours after admission.

An autopsy was made three hours post mortem. The body was that of a poorly nourished Negro girl about 2½ months old.

The pericardial sac contained a normal amount of straw-colored fluid. The visceral pericardium appeared slightly glazed. The heart weighed 50 Gm. The right and left ventricular walls were thickened, with marked dilatation of both ventricular chambers.

The trabeculae carneae, papillary muscles and chordae tendineae of the left ventricle had a bright yellow color. On section the entire myocardium was slightly softened and had a pale mottled yellowish pink appearance.

The upper lobes and the posterior portions of the lungs showed complete hemorrhagic solidification, with smaller firm reddish areas, devoid of air, scattered through the remainder of the lung substance.

The liver was intensely congested and showed yellowish discoloration of its substance, particularly about the central veins.

The brain showed an area of subarachnoid hemorrhage over the entire lateral aspect of the left frontoparietal region.

Microscopically, the visceral pericardium and the entire myocardium were heavily infiltrated by small lymphocytes and mononuclear phagocytes with occasional plasma cells, polymorphonuclear leukocytes and young fibroblasts (*A* in figure). In the outer portion of the myocardium this inflammatory exudate was most conspicuous in the fibrous tissue framework and about blood vessels. In its inner third the cellular reaction became more intense. There were typical granulomatous areas and foci of coagulation necrosis. Situated from 0.5 to 1 mm. beneath the endocardium of the left ventricle were multiple foci of necrosis. These areas were rounded, lozenge-shaped or linear. Some showed a coagulative type of necrosis and contained much nuclear and cytoplasmic debris; others presented a typical gummatous type of necrosis, containing a few faint silhouettes of cardiac muscle (*B* in figure). There was much degeneration of the cardiac muscle generally, with extreme vacuolation, fraying and loss of cross striations.

The entire mural endocardium was thickened, with swelling of its cellular constituents and roughening and erosion of the endothelium, on which in both ventricles were several small mural thrombi. The inflammatory infiltrate and areas of necrosis were identical with those in the myocardium. At least one definitely gummatous area extended into the endocardium.

The veins within the myocardium showed marked thickening of their walls, with intimal hyperplasia and heavy infiltration by lymphocytes and plasma cells so as nearly to obliterate their lumens.

The gummatous areas of necrosis were most abundant in the trabeculae carneae of the left ventricle, in the anterior and posterior papillary muscles of the mitral valve and in the myocardium about the bases of these muscles.

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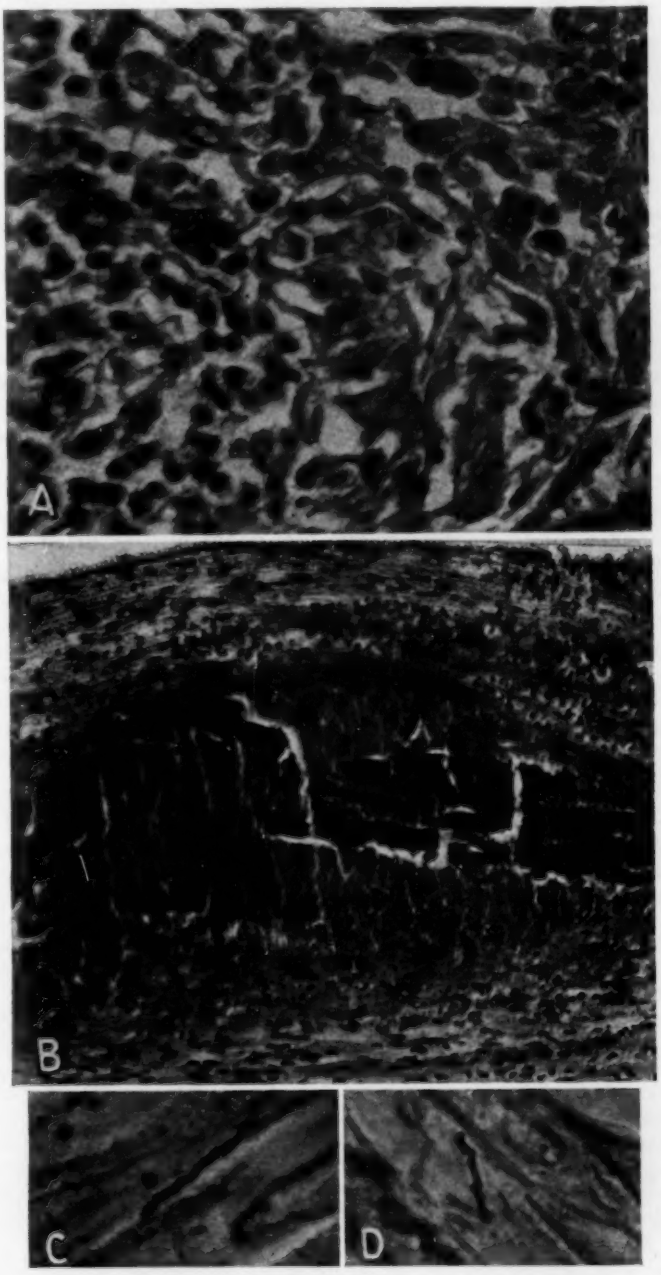
Levaditi stains revealed Spirochaeta pallida in the myocardium, most abundant adjacent to granulomatous and gummatous foci (*C* in figure). Four organisms were the greatest number ever found in one oil immersion field.



A, infiltration of the myocardium by plasma cells, histiocytes and lymphocytes. Hematoxylin and eosin; × 600.

B, gummatous area of necrosis beneath the endocardium of the left ventricle. Hematoxylin and eosin; × 130.

C and *D*, Spirochaeta pallida in the myocardium. Levaditi; × 2500.

The lungs showed the exudate of acute pneumonia in an early stage, and many of the alveoli were completely filled with erythrocytes and edema fluid.

In the leptomeninges, in the area noted grossly there was evidence of recent hemorrhage.

No evidence of syphilis was found in any organ except the heart.

COMMENT

The myocardial lesion described represents one of the rarer forms of myocardial syphilis, namely, a diffuse gummatous process unaccompanied by gross gummas. Sohval [1] was able to find only 7 similar cases in the literature. He collected 97 cases of grossly recognizable cardiac gumma, however, and added 2 of his own. Von Haam and Ogden [2] reported 3 additional cases of gumma of the heart, the first being one of isolated gummatous pericarditis. Braunstein and Bass [3] described a case of multiple cardiac gummas with aneurysm of the left ventricular myocardium. The addition of these cases brings the total number of cases of gross cardiac gumma reported to 101. In none of the recently reported cases was there any example of diffuse gummatous myocarditis, without macroscopic gummas, although in the third case of von Haam and Ogden such a picture was closely simulated, as the extensive gummatous myocarditis was accompanied by only one small gross gumma.

It is essential that certain definite histologic criteria be applied in making the diagnosis of gummatous myocarditis. These morphologic features, excellently summarized by Sohval,[1] are as follows: widespread perivascular and interstitial granulation tissue infiltrated by lymphocytes and plasma cells; proliferative and often obliterative endovascular lesions, and areas of coagulation necrosis varying from submiliary foci to a size approaching that of the well localized gumma. Remnants of myocardial fibers in the areas of necrosis aid in differentiating gummatous processes from tuberculous ones. A number of reported cases of cardiac gumma have not fulfilled the diagnostic criteria or have lacked adequate description. In some of them the lesions were undoubtedly of syphilitic nature but not truly gummatous. Williams [4] cited some instances of this type and reported the case of a newborn Negro girl whose heart contained three nodules which were grossly consistent with gummas but which failed on microscopic examination to show the characteristic features and are simply best described as syphilitic myocarditis.

SUMMARY

A case of diffuse syphilitic myocarditis in an infant with congenital syphilis is reported. There were multiple microscopic areas of gummatous necrosis. Only 7 other cases of a similar nature have been recorded.

1. Sohval, A. R.: Arch. Path. **20**:429, 1935.
2. von Haam, E., and Ogden, M. A.: Arch. Path. **26**:525, 1938.
3. Braunstein, A. L.; Bass, J. B., and Thomas, S.: Am. Heart J. **19**:613, 1940.
4. Williams, J. W.: Am. J. Path. **6**:573, 1930.

CHONDROSARCOMA OF THE NASOPHARYNX SIMULATING JUVENILE ANGIOFIBROMA

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AND
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Chondrosarcoma of the nasopharynx is a rarity. Schmidtmann [1] stated that the incidence of sarcoma in this site is approximately 0.03 per cent and that chondrosarcoma accounts for a small percentage of the group. We record here the history of a 16 year old boy with a tumor of the nasopharynx, which was originally diagnosed as juvenile angiofibroma but which on subsequent course and termination proved to be chondrosarcoma. The tumor grew progressively despite treatment with radon and partial excisions, penetrated into the cranial cavity and metastasized to the lungs. The patient's progress was followed for three years, periodic biopsies of the tumor were done, and a necropsy was performed.

REPORT OF A CASE

A 16 year old white boy was admitted to the Tumor Clinic of the Marine Hospital, in Baltimore, on Jan. 2, 1940, with the history of complete obstruction of the left side of the nose and a lump in the roof of the mouth of about four months' duration. He had no pain or discomfort other than inability to breathe through the left naris.

The patient's past history and the family history were irrelevant. Both parents and two brothers were living and well. The patient had his tonsils removed as a child.

He was a small, slight adolescent boy, 64 inches (162.5 cm.) tall and weighing 96 pounds (43.5 Kg.). The entire left side of the nasopharynx was filled with a firm, resilient, rounded mass. The tumor also filled the entire posterior part of the left naris, pushing the septum to the right and protruding anteriorly so that it pushed the turbinates anteriorly and medially. The growth seemed to rise from the lateral pharyngeal wall in the region of the fossa of Rosenmüller. It distorted this structure so that it could hardly be identified. Superiorly the tumor extended to the roof of the nasopharynx, medially to the midline and inferiorly to the superior pole of the tonsil. It also encroached on the soft palate, pushing it downward into the throat for a distance of 1 cm. The mass could not be seen through the naris because of the distorted, overlapping turbinates. The tumor was covered by pale mucosa with branching dilated blood vessels on the surface. There was no ulceration, and the consistency was approximately the same throughout. The left ear drum was retracted, and the hearing was diminished on that side, with bone conduction unimpaired.

The urine and the blood revealed no abnormalities. The Kline and Eagle tests were negative. Roentgenograms of the skull showed no definite involvement of bone, and the left antrum was clear.

The mucosa of the soft palate was incised until the rounded encapsulated firm edges of the tumor were encountered, and a wedge-shaped piece of tissue was removed.

Biopsy showed a moderately cellular tumor which was broken up into small islands by endothelium-lined vessels. The tumor cells had fairly small, irregularly shaped nuclei and a little clear cytoplasm. No mitotic figures were seen. There were areas of collagen production, particularly at the periphery of the tumor. Several small areas of hyalin-like substance, apparently cartilage, were present. The tumor terminated at a fibrous capsule and was not infiltrative (fig. 1 *A* and *B*).

The clinical diagnosis, apparently substantiated by the biopsy, was juvenile angiofibroma of the nasopharynx.

From the Tumor Clinic, Marine Hospital.

1. Schmidtmann, M.: Stutzsubstanzgeschwülste der Nase und Nebenhöhlen, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1928, vol. 3, pt. 1, p. 232.

In January the patient was given, during the course of eight days, three 250 kilovolt (peak) roentgen ray treatments to the left side of the nasopharynx, totaling 700 roentgens (r), through the antral portal and two 140 kilovolt (peak) roentgen ray treatments, totaling 418 r, through the mouth. The tumor did not respond to this therapy. Beginning in April 1940 and continuing at approximately semimonthly intervals until January 1941, radon in gold tubes [2] was implanted in the tumor. In all, 40 tubes containing 24.5 millicuries of radon were implanted.

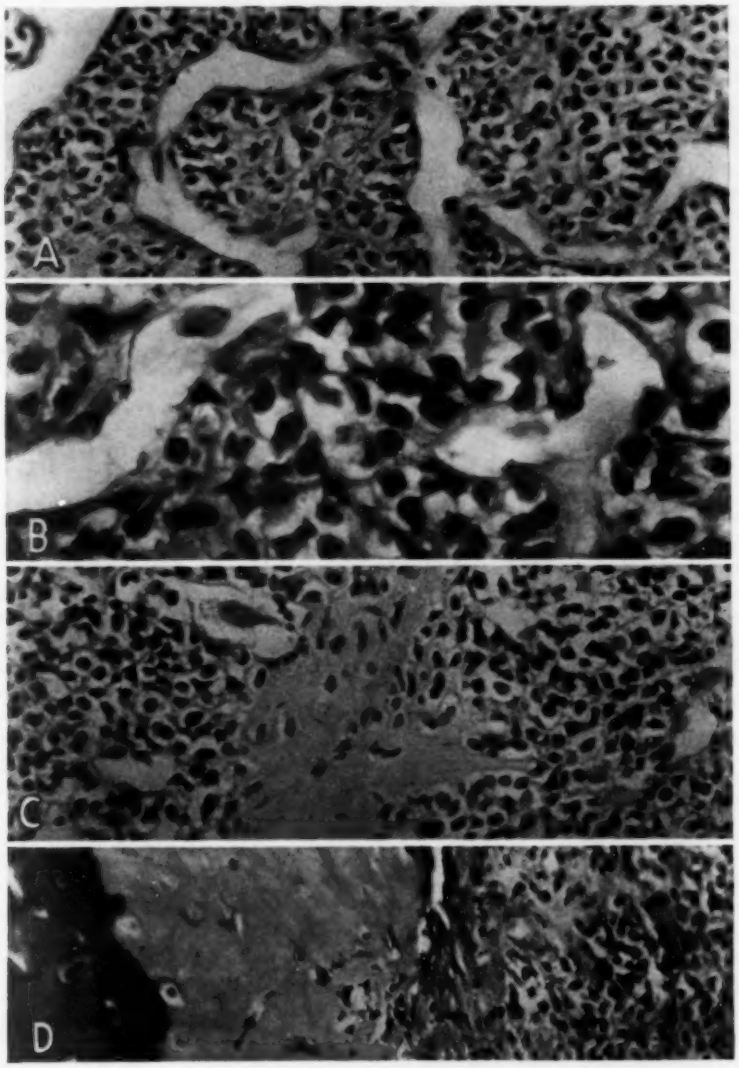


Fig. 1.—*A*, biopsy section of Jan. 3, 1940. A diagnosis of juvenile angiofibroma was made on the basis of the fibroblastic cells separated by prominent endothelium-lined vessels. Hematoxylin and eosin; × 335. *B*, higher magnification (× 1,480) of *A*. *C*, biopsy section of Oct. 16, 1941. The tissue was more cellular, the blood sinuses were less pronounced, and there were areas of cartilage production. Hematoxylin and eosin; × 335. *D*, biopsy section of Nov. 26, 1941. The sections showed extensive areas of cartilage and bone formation. Hematoxylin and eosin; × 270.

2. The length was 2.5 mm.; the wall thickness, 0.3 mm.

In March 1941 examination showed that the mass had become harder and had shrunk upward. The posterior tonsillar pillar on the left side was now visible as a distinct structure. Posteriorly in the naris, the mass appeared as a rounded structure separate from the turbinate, and the fossa of Rosenmüller was no longer deformed by the mass. Looking through the nose, one could discern the mass anteriorly under the inferior turbinate and superiorly above and behind the middle turbinate.

From May to August 1941, 25 millicuries of radon in 24 gold tubes was implanted. In July check-up films of the skull showed slightly increased expansion of the mass laterally. The patient complained of pain and discomfort in the left side of his jaw on mastication. In September he had diplopia for a few days, and in October he was brought to the hospital because of protrusion of the left eye and pain and swelling of the left side of the face. There was paralysis of the external rectus muscle of the left eye. Roentgenograms of the skull showed increased density in the left antrum and the left ethmoid cells. No significant changes were seen in the skull or in the outline of the left orbit. Review of previous roentgenograms showed that the left antrum was definitely clear in July but that even in the films made Jan. 30, 1941 there was a suggestion of a break in the lateral wall of the left portion of the ethmoid.

October 15 diagnostic antrotomy was performed. The entire left antrum was filled with a mass of polypoid tumor, which destroyed a portion of the medial wall and the roof of the antrum. The swelling in the left side of the face subsided slowly, but ten days after the operation there was already recurrence of tumor nodules. The patient continued to have diplopia owing to partial paralysis of the left external rectus muscle.

Microscopic examination of the tissue removed from the antrum showed a uniform cellular structure split into fairly regular lobules and islands by numerous endothelium-lined channels. The cells within the lobules were arranged in rows, were of moderate size, had clear cytoplasm and appeared to be producing fine connective tissue stroma. There were no mitotic figures, and the general structure was fairly regular. In comparison with the specimen taken for biopsy in January 1940, the tissue was more cellular and produced less collagen, there was less regularity of the cells, there were more areas of cartilage formation, and the blood sinuses were less pronounced (fig. 1*C*). The diagnosis was vascular embryonal chondroma.

November 26, after preliminary tracheotomy and ligation of the left external carotid artery, the major portion of the mass in the left side of the nasopharynx, antrum, pharyngeal wall and pterygoid fossa was removed.

Microscopic examination of the tissue showed a dense growth of connective tissue which was split into small lobules by vascular channels. The tissue merged into cartilage which contained small areas of calcification and bone formation (fig. 1*D*).

Examination December 10 showed residual tumor in the region of the sphenoid sinus and in the nose just above the middle turbinate. With the patient under anesthesia induced with pentothal sodium, a catheter was passed through the left naris above this mass and drawn downward. The mass measured over 2 cm. in diameter and was wedged tightly between the septum and the region of the left orbit. It was removed by gross dissection.

In January 1942 the entire right side of the nasopharynx and fossa of Rosenmüller were clear, and the mucous membrane was intact. The roof of the nasopharynx on the left also appeared free of disease. The left choana was now completely visible. The left inferior turbinate, however, was surrounded by a small globular mass of tumor.

An attempt to remove this mass was made January 7. Small bits of tissue were removed from the medial and posterosuperior walls of the antrum, and were shown to consist of residual tumor as well as of small chips of bone and cartilage.

Postoperatively, pain developed in the left ear, and there was bulging of the superior half of the drum. The temperature rose to 40 C. (104 F.). Myringotomy produced only profuse bleeding from the floor of the canal. Roentgenograms of the left mastoid bone showed increased density of the cells. The patient was given eighteen daily 250 kilovolt (peak) roentgen ray treatments for a total dose of 1,425 r, to the left ear with noticeable subjective improvement. He gained some weight, and a prosthesis was designed to cover the defect in the palate.

In April 1942 he was readmitted because of numerous small hemorrhages from the posterior wall of the pharynx. May 4, 1942, after preliminary tracheotomy, the anterior wall of the left antrum, the entire infraorbital ridge, the left eye and the intraorbital contents were removed. The tumor had refilled the antrum and had extended from the orbital medial wall backward to the sphenoid bone. The tumor had apparently broken through the posterior wall of the left side of the sphenoid sinus to extend down along the entire base of the skull from the midline outward to the left into the pterygoid fossa and down the posterior pharyngeal wall from

the midline to the left into the tonsillar fossa, where it formed a lateral retropharyngeal mass 2 cm. in diameter and protruded 1 cm. into the pharynx. As much of the tumor as possible was removed.

Microscopic examination of the tissue revealed areas of well differentiated fibroblastic cells separated by vascular channels. There were also areas of cartilage and spicules of bone formation. Portions of the tumor, however, were now much more cellular, showed less fibrous connective tissue stroma, and the cells were spindle shaped. Among these less differentiated spindle-shaped cells were occasional mitotic figures and small groups of cells with bizarre large nuclei (fig. 2*A*). The diagnosis of chondrosarcoma was made.

Postoperatively, a small retropharyngeal abscess developed, and there was a severe hemorrhage from the orbital artery. There was extreme pain throughout the head and left ear.

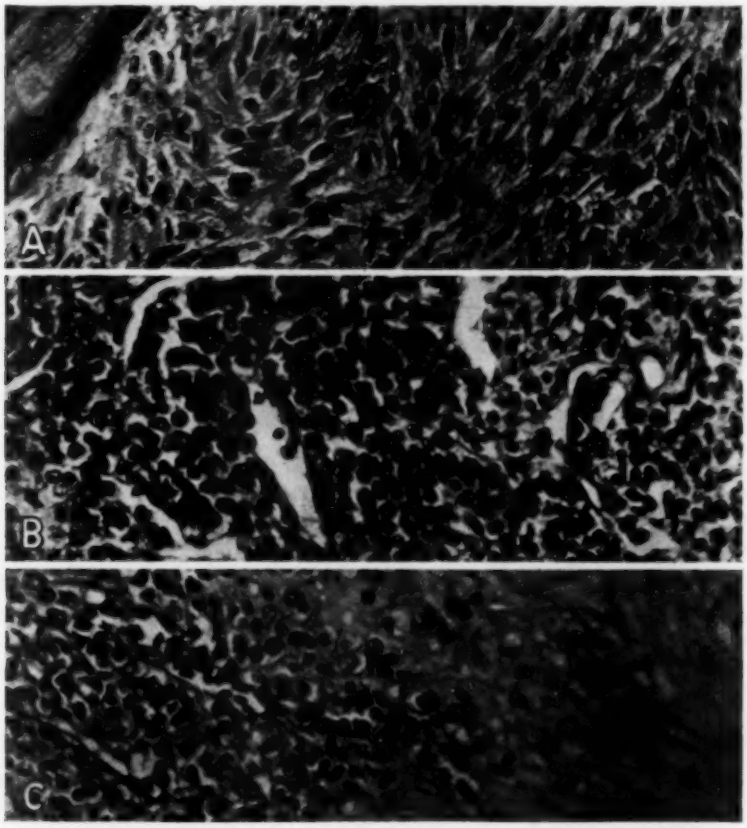


Fig. 2.—*A*, biopsy tissue of May 4, 1942. The sections showed greater cellularity. There were spindle-shaped cells, occasional mitotic figures and cells with bizarre nuclei. Small areas of cartilage and bone spicules were present. Hematoxylin and eosin; × 270. *B*, necropsy section of tumor that had penetrated into the cranial cavity, showing a cellular tumor with many blood channels. Hematoxylin and eosin; × 335. *C*, section of a pulmonary nodule at the border of the cellular and the cartilaginous areas; × 335.

July 20, as an attempt at palliation, 11 millicuries of radon in 11 gold tubes was implanted into the residual mass in the left lateral pharyngeal wall.

In September 1942 the patient complained that he could not see from the side of his right eye. Examination showed paralysis of the right external rectus muscle.

October 5, 6.7 millicuries of radon in 16 gold tubes was inserted throughout the posterior upper part of the left alveolus and the region of the anterior tonsillar pillar. The patient continued to have pain throughout the entire head and severe hemorrhages, apparently from the sphenoid region. He began to lose weight and strength. November 3 roentgenograms showed multiple small nodules scattered throughout the lungs.

He became progressively weaker and more emaciated. Roentgenograms of the chest showed rapid extension of the disease in the lungs. Despite sedation with morphine, pain in the head was intense. He died Jan. 3, 1943, approximately three and a half years after the onset of his illness.

Necropsy.—The body was thin and wasted, 67 inches (170 cm.) long and about 80 pounds (36 Kg.) in weight. There was no facial hair, and the pubic hair was feminine in distribution. The left eye had been enucleated, and the cavity communicated with the left maxillary sinus. The base of this cavity was lined with hard, grayish white nodular tissue. The superficial lymph nodes were not enlarged.

The medial cranial fossa on the left was replaced by a subdural tumor which completely destroyed the floor of the cranium in this region and protruded into the cranial space in the form of two adherent nodular masses, each about 4 cm. in diameter. The tumor was adherent to the dura but penetrated it only at two small points at the anterior and the posterior periphery. These single papillary nodules of tumor, about 3 mm. in diameter, projected through the dura. The left temporal lobe of the brain was compressed and atrophied.

The mass also destroyed the sphenoid process and the sella turcica on the left, completely filling the region and destroying the bone. The pituitary gland and the optic nerves were lifted by the tumor. The extent of the tumor made it impossible to determine the site of origin; the incompletely removed tumor mass weighed 100 Gm.

Except for the compression atrophy of the left temporal lobe, the brain was normal.

The cervical lymph nodes and the thyroid gland were normal.

The right lung weighed 520 Gm. and the left lung 220 Gm. Both lungs contained multiple hard round masses which on palpation felt like marbles and were slightly movable. The nodules slipped away from the knife when the tissue was sectioned. They were easily enucleated from the lung, were firm, gritty and whitish gray, with somewhat translucent centers, and measured from a few millimeters to 2 or 3 cm. in diameter. The masses were scattered evenly throughout the lung substance, a few lying directly under the pleura and some near the hilar regions. In all, considerably more than 100 discrete nodules were present.

The lower lobe of the right lung was consolidated, and there were small areas of pneumonic consolidation at the base of the left lung. Present at the bases were some recent fibrous adhesions of the pleura. Lying above the right bronchus was one enlarged mediastinal lymph node.

The remainder of the necropsy revealed no abnormalities except generalized atrophy. The heart weighed 110 Gm.; the spleen, 60 Gm.; the liver, 1,020 Gm., and the kidneys 90 Gm. each.

Microscopic Examination.—Sections of the tumor that had penetrated into the cranial cavity showed the growth to be highly cellular, the spindle-like cells having oval hyperchromatic nuclei and scanty cytoplasm. There was considerable variation in size and shape, and occasional mitotic figures were encountered (fig. 2*B*). In some areas the tumor produced cartilage. The angiomatous features were not marked, the blood vessels being compressed and relatively few. The tumor did not penetrate the dura, but the lumens of some blood vessels on the dura contained small plugs of tumor tissue, and several small vessels were surrounded by the tumor, which infiltrated the vessel wall.

In the lung the metastatic nodules consisted of the same type of tumor tissue. In the center of these nodes there was considerable production of cartilage as well as areas of calcification. Toward the periphery the cellularity became more extensive (fig. 2*C*). As in the cranial tumor, the blood channels were not prominent. The nodules appeared to have a sharp border of thin fibrous tissue and compressed rather than invaded the lung. In other areas, however, the tumor was not delineated so sharply at its periphery and was much more invasive. Some blood vessels in the lung were seen to contain tumor thrombi. The lung showed marked infiltration by polymorphonuclear and red blood cells, and fibrinous exudate filled the alveoli in a spotty manner. Purulent material was present also in the bronchioles.

The mediastinal lymph nodes were free of tumor. The testes showed atrophy of the tubules. The spleen showed atrophic follicles. The kidneys, adrenal glands, pancreas, thyroid gland, and pituitary gland appeared normal.

Diagnosis.—Chondrosarcoma of the nasopharynx with penetration into the cranial cavity and metastases to the lungs; lobular pneumonia.

COMMENT

Clinically this case was of interest because the nasopharyngeal chondrosarcoma was confused with a more common tumor occurring in adolescent males, the juvenile angiofibroma. The latter tumor, which does not metastasize and often regresses spontaneously at the end of the period of bodily development and growth, originates most commonly from the fascia basalis of the occipital bone and the body of the

sphenoid bone. The original biopsy tissue appeared compatible with this diagnosis. The lack of response to radon therapy, which is effective in juvenile angiofibroma,[3] and the subsequent biopsies of the tumor, however, showed the growth to be chondromatous. In retrospect, it is probable that the original sections consisted only of the cellular peripheral portion of the tumor. Small areas of osteogenesis and chondrogenesis are encountered occasionally in the tumors classed as angiofibroma.[4] Later sections were obtained from the more central portions where, as in the pulmonary metastases, the chondromatous nature of the growth was much more apparent.

The earlier literature on the subject does not differentiate the juvenile angiofibroma as sharply from similar tumors of this region as the recent comprehensive reviews of Brunner,[4] Figi [3] and others. Coenen,[5] for example, stated that there was little difference between the benign fibroid and the chondrosarcoma and the sarcoma arising from the base of the skull. Earlier reports of cancerous transformation of juvenile fibroma are questionable, since the criteria for such change were rapid growth, destruction of contiguous tissues and local recurrence after surgical removal, now stated to be characteristic of the "benign" tumors. There appears little doubt that the juvenile angiofibroma is quite distinct from the type of tumor reported here.

The size of the tumor at the original examination precluded the possibility of establishing the exact point of origin. The penetration of the lateral wall of the left portion of the ethmoid, seen in roentgenograms of the skull in January 1941, suggests, however, that the tumor arose in the ethmoid region. This point of origin would clarify the cartilaginous nature of the tumor, since the ethmoid and the septum of the nose are derived from the ethmovomerine cartilage of the chondrocranium. It is known, however, that cartilage can develop from the periosteum of membrane bone [6] or extraskeletally.[7] The tumor recorded here could have arisen, therefore, from the base of the sphenoid bone rather than from the ethmoid or the nasal septum. The serial roentgenograms of the skull indicate that the left maxillary sinus was invaded by the tumor rather than that it was the site of origin.

The morphologic character of the pulmonary metastases made certain the diagnosis of chondrosarcoma. In the skull the tumor was composed chiefly of primitive fibroblasts, with areas of round cells, which Geschickter and Copeland [8] consider to be midway between fetal cartilage and chondroblasts in differentiation. In the lungs there was a reproduction of the embryonal development of bone, from the densely cellular periphery of fibroblasts and increasing amounts of hyalin-like material to the central portion of cartilage and calcification.

Chondrosarcoma is radioresistant,[8] and in this case rather extensive treatment with radon did not influence markedly the progress of its growth. The only possible effective therapy would have been early radical dissection, which was perhaps impossible even when the patient was first seen, and with a clinical and histologic diagnosis of juvenile angiofibroma was not indicated.

SUMMARY

A case of chondrosarcoma of the nasopharynx in a boy 16 years of age is reported. The tumor invaded the cranial cavity and metastasized to the lungs.

3. Figi, F. A.: J. A. M. A. **115**:665, 1940.
4. Brunner, H.: Ann. Otol., Rhin. & Laryng. **51**:26, 1942.
5. Coenen, H.: München. med. Wchnschr. **70**:829, 1923.
6. Pasternack, J. G.; Lillie, R. D., and Jones, R. A.: Arch. Path. **15**:649, 1933.
7. Binkley, J. S., and Stewart, F. W.: Arch. Path. **29**:42, 1940.
8. Geschickter, C. F., and Copeland, M. M.: Tumors of Bone, New York, American Journal of Cancer, 1931.

EXTRAMEDULLARY PLASMA CELL TUMOR OF A TONSIL WITH METASTASIS

W. L. McNamara, M.D., and R. J. Rogers, M.D., Hines, Ill.

Mattick and Thibaudeau [1] noted that "the occurrence of plasma cell tumors of extramedullary origin is of sufficient interest and rarity to merit report of even a single case."

A white clergyman, aged 60, entered the tumor service Jan. 13, 1941, complaining of a lump in the right side of the neck and difficulty in swallowing (fig. 1). The family history was essentially irrelative. The patient had had the usual childhood diseases. He considered his health good until about eight years ago, when a tumor of the right tonsil was noted. This was removed by the family physician about two years later, when it had reached the size of a walnut. It was diagnosed as carcinoma by one physician and as lymphosarcoma by another. At this time there were no masses in the neck. About one year later a small lump was noted in the right side of the neck which was painless and which gradually increased

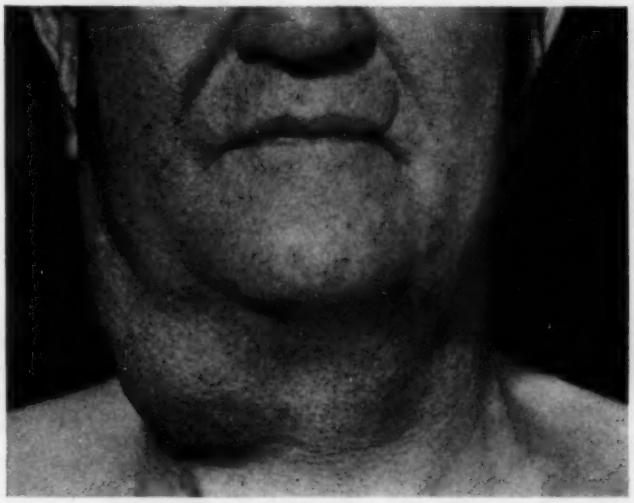


Fig. 1.—Photograph of the metastatic mass in the right cervical region.

in size until at the time of admission, six years after removal of the original tumor, it had reached the size of an orange and caused difficulty in swallowing. The patient had not lost weight appreciably.

He was well developed and well nourished, weighing 175 pounds (79.5 Kg.). The heart and the lungs revealed nothing of note. The blood pressure was 130 systolic and 80 diastolic. Routine laboratory tests, including Wassermann and Kahn tests, were negative. The eyes, the ears and the nose revealed nothing of interest.

The right tonsil had been removed. The left tonsil appeared normal. There was no evidence of recurrence of any lesion in the right tonsillar bed, in the throat or in the mouth.

The mass in the right cervical region extended from the inferior angle of the mandible almost to the base of the neck. Posteriorly it extended to the posterior border of the sternocleidomastoid muscle, and anteriorly, to within 3 cm. of the thyroid notch. It was firm but not hard. The overlying skin was free. Whether it was fixed to the deeper structures was difficult to determine because of its size; when it was manipulated, all the structures on that

From the Pathological Laboratory and Tumor Service of the Veterans Administration Facility.

1. Mattick, W. L., and Thibaudeau, A. A.: Am. J. Cancer **23**:513, 1935.

side seemed to move. In some areas it seemed fluctuant, but this could not be determined definitely. Palpation did not elicit any tenderness.

January 21 a small piece of tissue removed from the cervical mass showed on microscopic examination no normal lymph node structure except for an occasional small lymph follicle. The tissue for the most part was made up of cells with an occasional large multinucleate cell. The cells were round or oval with round, eccentrically placed nuclei (fig. 2). The chromatin in the nuclei consisted of coarse granules, irregularly placed; in many nuclei it was arranged along the nuclear membrane. The cytoplasm was profuse, opaque and nongranular; it stained bluish red with hematoxylin and eosin. No mitotic figures were seen. Morphologically, these cells were identical with the cells of plasma cell myeloma of bone.

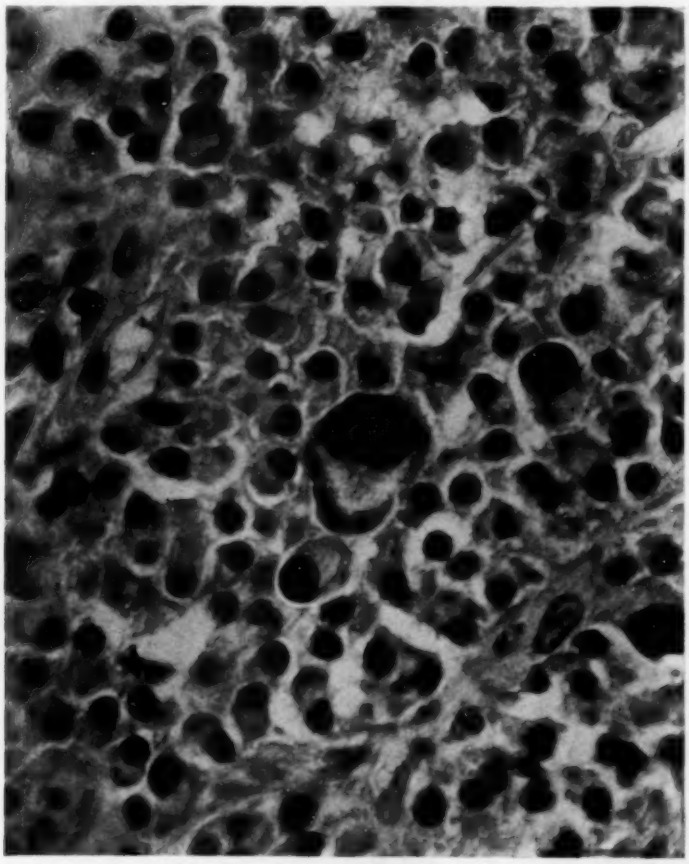


Fig. 2.—High power photomicrograph of plasma cells in a section from the cervical metastasis.

A section from the original tonsillar tumor removed six years previously was found to be identical histologically with the specimen from the cervical mass.

The tumor proved to be markedly radiosensitive. Between January 27 and February 17 2,500 roentgens of high voltage roentgen rays was given, and the mass shrank to a small fraction of its former size.

While on leave, March 19, 1941, the patient died suddenly. No autopsy was made.

SUMMARY

An instance of plasma cell tumor of the right tonsil with extensive cervical metastasis is reported. The clinical course was prolonged. The cervical metastasis was highly radiosensitive.

CONGENITAL DEFECT IN THE MUSCULATURE OF THE STOMACH WITH RUPTURE IN A NEWBORN INFANT

PETER A. HERBUT, M.D., PHILADELPHIA

Perforation of the stomach of the newborn infant is rare. In 1934 Dunham and Goldstein [1] collected 9 cases from the literature and added 2 others. A case not included in their series was published by Yovanowitch and associates in 1927.[2] Subsequently Smythe [3] reported 2 cases and Tow and Ross [4] reported 1 case, bringing the total to 15. The following is the sixteenth case to be put on record. It is unique because, as a careful search of the medical literature discloses, it is the first in which a congenital defect in the musculature of the stomach wall has been described and because it is the first in which rupture has occurred for want of such a covering.

A Negro boy 5 days old was admitted to the pediatric service of Dr. E. L. Bauer with a history of vomiting greenish yellow material and of having a distended abdomen, both of one day's duration. Following an uneventful spontaneous delivery at term, the infant nursed at the breast normally for the first two days. On the third day he was less active than previously, nursed with less enthusiasm and vomited greenish yellow material on one occasion. On the fourth day he tried to nurse but was unsuccessful because of repeated vomiting. There was a markedly distended and tense abdomen. Bowel movements, mostly of meconium, were normal. There was no evidence of pneumonia. The temperature was 98.8 F. The respirations were labored and numbered 50 per minute. The blood count was normal. Vomiting of greenish yellow material persisted. Attempts to relieve the abdominal distention by means of enemas and a stomach tube were only temporarily successful. Within a few hours after admission, the temperature rose to 100 F. and the respirations to 86 per minute, and the infant died.

At necropsy, when the abdomen was opened, air was heard and felt to gush past the incision. The peritoneal cavity contained about 100 cc. of gray watery exudate sprinkled with gray flecks. The stomach was in the normal position. It measured 4 cm. along the lesser curvature and 10.5 cm. along the greater curvature. About the midportion of the greater curvature, 0.6 cm. superior to the attachment of the omentum, the entire muscle of the anterior stomach wall was absent over an elliptic area, 6 by 3.5 cm. (fig.). At both the proximal and the distal end the merging bands of muscle united in the form of a V. The free edges of the muscle were round and smooth and closely adherent to the underlying tissue. They were covered with considerable gray watery exudate. The entire defect was filled with thin, somewhat indurated mucosa, varying in color from pink to gray, the central portion of which contained an irregular rupture about 4.5 cm. long. The inner surface of the mucosa was otherwise intact throughout. There were two small defects in the musculature of the posterior wall of the stomach. The proximal one was situated opposite the midportion of the greater curvature, 0.5 cm. superior to the omental attachment, and measured 0.6 by 0.3 cm. in diameter. The distal one was 0.6 cm. nearer the pylorus and 0.7 cm. superior to the attachment of the omentum; it measured 0.4 cm. in diameter. The serosal covering over each was smooth, intact and seemingly free of exudate. The edges were sharp but somewhat more sloping than those of the large anterior defect. The central portions were completely devoid of muscle tissue. The exposed intact gray submucosa was easily seen through the transparent serosal covering. The omentum was short, thick, dark red to orange yellow and firm. It was not attached to any of the surrounding structures. The entire gastrointestinal tract beyond the defects was carefully examined for obstruction, but none was found. The lower part of the ileum, the cecum and the ascending colon were mobile.

From the Clinical Laboratories, Jefferson Medical College Hospital.

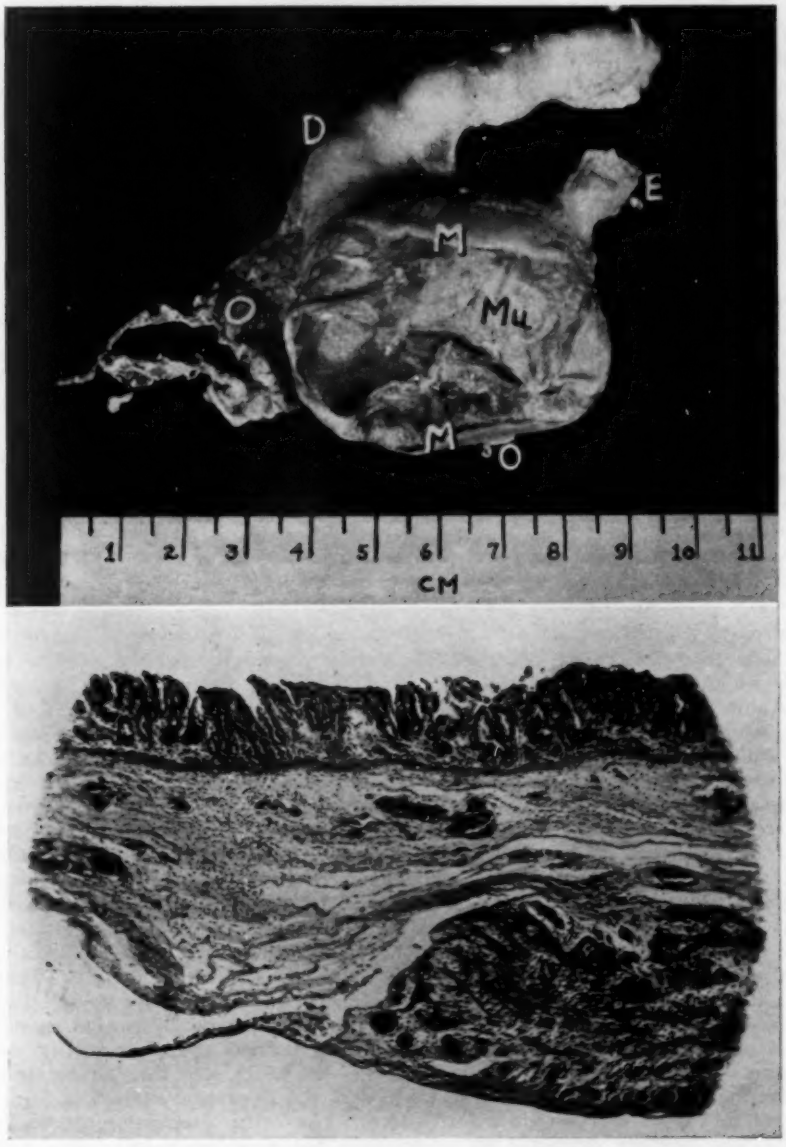
1. Dunham, E. C., and Goldstein, R. M.: J. Pediat. **4**:44, 1934.

2. Yovanowitch; Kuhlmann; Weiss, and Woringer: J. de méd. de Paris **46**:774, 1927.

3. Smythe, F. W.: Am. J. Surg. **24**:818, 1934.

4. Tow, A., and Ross, H.: J. A. M. A. **111**:1178, 1938.

Microscopic examination of the gastric wall at the reflection of the muscle in each of the three defects disclosed normal mucosa, mucosa submuscularis and submucosa (fig.). The muscle ended abruptly and completely. In sections from the defects in the posterior wall the normal serosa extended beyond the free border of the muscle, completely covering the denuded submucosa. The entire external surface of the defect in the anterior wall was covered with an exudate of nuclear fragments, polymorphonuclear leukocytes and some fibrin.



Upper: Anterior aspect of a newborn infant's stomach and neighboring structures, showing the edges of normal muscle (*M*), an irregular rupture of the bare mucosa (*Mu*), the attached omentum (*O*), the esophagus (*E*) and the duodenum (*D*).

Lower: Microscopic section through a defect of the posterior wall of the stomach showing the intact mucosa, mucosa submuscularis and submucosa, the abrupt and complete termination of the musculature and the intact serosa with its mesothelial layer of cells covering both the muscle coats and the denuded submucosa. The separation in the lower left hand corner is an artefact. Hematoxylin and eosin; × 50.

This was so marked that the serosa, covering both the normal portions of the wall and the exposed submucosa, was obscured and could not be absolutely identified, although in many sections its presence was strongly suggested.

COMMENT

In 14 of the 15 previously reported cases the rupture of the stomach in the newborn infant was secondary to a gastric ulcer. In the remaining case there was no ulceration, the borders of the perforated area showing only hemorrhages. In the case reported here, there likewise was no ulceration. Because each of the defects was bridged by normal gastric mucosa, mucosa submuscularis and submucosa and particularly because in the defects of the posterior wall an intact serosal covering was definitely identified, each lesion was considered as primarily an absence of the muscle layers. Rupture of the unsupported mucosa of the anterior wall probably occurred after food was ingested.

Although there is no doubt that the abnormalities were of congenital origin, their genesis is somewhat difficult to ascertain. In man the entire gastrointestinal tract is formed from mesenchymal cells arising from the inner cell mass.[5] In the development of the stomach the differentiation of the musculature, as demonstrated by Plenk,[6] is first discernible in the 11 mm. embryo. It occurs as a thickening of the mesenchyme at the lower end of the esophagus and along the lesser curvature of the stomach and forms the anlage for the circular muscle layer. Later, in the 13 mm. embryo, there are similar thickenings in the fundus and at the greater curvature. The longitudinal muscle fibers appear next. They are always very thin, and even at birth are in part entirely absent. The oblique fibers are first discernible in the 9 week embryo and arise from the circular layer.

In the case reported here it appears that the development of the gastric musculature alone was defective. Because the defects were located along the greater curvature, it is probable that the anlage at the lower end of the esophagus and along the lesser curvature developed normally, while the thickenings of the fundus did not. Since the posterior defects were small, failure to cover the entire wall must have been a terminal arrest of development. On the other hand, because the anterior defect was large, the mesenchymal thickenings of the fundus and anterior wall were either poorly developed or failed to appear. In either case the growth of the muscle cells from the primary anlage of the lesser curvature was not rapid enough to cover the normally developing mucosa and submucosa. The longitudinal fibers, which at best are very thin and in areas altogether absent, were not sufficient to provide an adequate covering or, perhaps, failed to appear at all.

In a recent review of the literature on spontaneous rupture of the intestine of the newborn infant Russell[7] collected a total of 39 cases. In 13 of these rupture occurred proximal to an obstruction, while in 26 there was no obstruction. In 28 cases perforation occurred during birth and, in his opinion, was due to pressure from the birth canal acting on a bowel filled with meconium. In a review of the literature on subcutaneous rupture of the stomach of the adult, Wolf[8] collected 17 cases of so-called spontaneous rupture. With regard to the mechanism of rupture, he expressed the belief that the force acts from within the viscus and that distention of the stomach by food either paralyzes the nervous mechanism, causing further dilatation, fermentation and eventually rupture, or interferes with the circulation, producing softening of the wall and subsequent perforation. Although it cannot be denied that these mechanisms play an important role, the basic underlying factor both in infants and in adults might well

5. Arey, L. B.: Developmental Anatomy: A Text Book and Laboratory Manual of Embryology, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 76.

6. Plenk, H.: Ztschr. f. mikr.-anat. Forsch. **26**:547, 1931.

7. Russell, T. H.: Tr. New England S. Soc. (1939) **22**:286, 1940.

8. Wolf, N. J.: New York State J. Med. **36**:1539, 1936.

be some small congenital defect in the muscle layers. This, of course, applies particularly to those cases in which there is no obstruction distal to the rupture. Otherwise, why should spontaneous perforation not occur more frequently?

Although surgical closure of the ruptured area in the newborn infant's stomach has been attempted, it has never been achieved. In any case of perforation of the gastrointestinal tract early diagnosis is imperative if the patient is to recover. In the cases previously reported, as well as in the one described here, there appears to have been a fairly constant, although not necessarily characteristic, group of signs and symptoms. Following an uneventful delivery, the infant is usually normal for the first one to four days. Then it may become apathetic, refuses its feedings, vomits, at first once or twice but later repeatedly, and finally shows distention of the abdomen and labored breathing. Bowel movements are usually normal. Occasionally the stools may contain blood. The diagnosis is definitely established when a flat roentgen plate of the abdomen discloses free air in the peritoneal cavity.

SUMMARY

The sixteenth case of perforation of the stomach of the newborn infant is described. It is the first case in which rupture occurred because of congenital absence of the muscle layers of the gastric wall.

General Reviews

EXTRAMEDULLARY PLASMA CELL TUMORS AS OBSERVED IN VARIOUS LOCATIONS

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WICHITA, KAN.

Tumors consisting of plasma cells occur quite frequently in the bone marrow, constituting one of the histologic types of multiple myeloma. The clinical and anatomic features of these tumors have been adequately discussed in general reviews and in textbooks. Extramedullary plasma cell tumors, on the other hand, are of greater rarity and have attracted much less attention. Articles on this interesting disease are with few exceptions reports of single cases, and since most of them have appeared in periodicals of surgical specialties or in foreign literature, they are not easily accessible to the tumor pathologist.

The present review is based on an analysis of all available articles on the extramedullary plasma cell tumor which appeared from 1905 until 1942. It comprises 127 published cases. In 63 of these the tumor originated in the air passages; in 47, in the conjunctiva; in 4, in lymph nodes, and in 13, in other organs (pleura, mediastinum, spermatic cord, thyroid gland, ovary, intestines, kidney and skin). A case of plasma cell tumor of the mouth which I had the opportunity to observe is included in this survey, making a total of 128 cases.

AIR PASSAGES AND MOUTH

The upper respiratory tract is the site of predilection for the extramedullary plasma cell tumor. The first tumor composed exclusively of plasma cells to be reported, that of Schridde's patient in 1905, was situated in the nasal cavity. The majority of the extramedullary plasma cell tumors reported since then have belonged in the field of the specialist in diseases of the nose and throat.

Following the general principles of tumor pathology, I have made an attempt, not always successful, to classify the available material into cancerous and noncancerous tumors.

Noncancerous Single Tumors.—In the literature are reported in detail 26 cases of noninvasive solitary plasma cell tumor of the upper air passages or of the oral cavity. There are sixteen reports of single cases; in a seventeenth paper 2 cases are described, and two authors, Ringertz and Menzel, had 4 cases each.

The youngest patient in this group was 40 years of age and the oldest 82. The average age was 56.6 years. It is interesting to note that of the 26 patients 23 were male.

As to the anatomic location of the tumors in this group, 9 were situated in the nasal cavity, 6 originating from the turbinates and 3 from the nasal septum or from the lateral wall. Six tumors were observed in the nasopharynx and 7 in the larynx. Three tumors originated from the tonsils and 3 from the fauces (pillar, uvula, soft palate), while 1 arose from the floor of the mouth. The tumor which I observed was situated under the tongue extending from the midline of the floor of the mouth to the right side of the lower jaw.

From the Department of Pathology, St. Francis Hospital, and the Sedgwick County Tumor Clinic.

Seven tumors in this group were described as polypoid or pedunculated, 15 as sessile and elevated and 3 as diffuse swellings of the mucosa. Their diameter varied from 5 mm. to 4 cm., the largest being in the nasal cavity and nasopharynx, which were often almost completely filled with tumor. The consistency was, as a rule, firm; the color, yellowish gray or bluish red. Eight tumors had a smooth and 3 a nodular surface; 4 showed superficial hemorrhage and 3 ulceration. The cut surface of most of these tumors was homogeneous and gray; few were distinctly lobulated.

Roentgen examination failed to show local invasion of bone, and there were no neoplastic processes in the regional lymph nodes or in distant bones.

Histologically, the abundance of plasma cells was the outstanding feature of these tumors. In typical cases, as in my own, the tumor cells were characterized by a large amount of basophilic cytoplasm and an eccentrically situated nucleus. The chromatin was arranged in cartwheel fashion, and a paranuclear unstained area was present. The size of the round, oval or polygonal plasma cell varied from 10 to 12 microns; that of the nucleus, from 4 to 6 microns. The cytoplasm gave a characteristic metachromatic reaction with Unna's polychrome methylene blue, while with Pappenheim's methyl green–pyronine the cytoplasm stained bright red and the nucleus bluish green.

Cells with two, three or more nuclei were not uncommon, and a certain variation in size of the cells and the nuclei was often observed. A large number of mitotic figures did not indicate malignancy since 3 tumors with many mitotic figures did not recur after excision.

The polyp-like plasma cell tumors had as a rule a more uniform cell structure than the sessile tumors and showed in sections stained with silver a much coarser reticulum. Some observers noted an abundance of capillaries and in the surrounding area of the tumor perivascular collars of plasma cells. Polymorphonuclear leukocytes, histiocytes and lymphocytes, if present at all, never formed an essential part of the tumor structure; Langhans and Sternberg-Reed giant cells were entirely absent. Degenerated plasma cells with red-stained globules, the so-called Russell bodies, were described by two observers, and were present in small number in the tumor which I observed.

While 23 tumors of the group were composed almost exclusively of typical plasma cells, the cell structure in 3 was more atypical, many of the tumor cells resembling lymphoblasts. Five tumors were regarded by their observers as cancerous on histologic grounds; however, after simple surgical removal no evidence of recurrence was noted for as long as eighteen years.

As to the clinical course of the solitary extramedullary plasma cell tumors of the noncancerous type, medical care was sought by most of the patients after one to two years of symptoms. These symptoms were almost entirely mechanical; nasal obstruction, difficulty in breathing, cough, hoarseness and the sensation of a foreign body in the throat were outstanding complaints. Seldom was pain or hemorrhage noticed, and the general condition was almost never affected. Serologic tests for syphilis, differential blood cell counts and a urinalysis gave, as a rule, normal results, and in no case were Bence Jones bodies found in the urine.

Most of the single noncancerous tumors, 19 in number, were removed surgically; 4 received radiation only, and 3 were given combined treatment. In most instances the follow-up period after treatment had been too short to allow definite conclusions. Only 4 patients were observed for longer than five years. Oppikofer's 46 year old patient with a plasma cell tumor of a tonsil showed no evidence of recurrence ten and one-half years after tonsillectomy. The same author ascer-

tained the late results in 2 cases which had been reported years before by Boit and von Werdt. Boit's patient, from whom a laryngeal plasma cell tumor was removed, was completely free from tumor eighteen years later, and von Werdt's patient, who was operated on for a tumor of the uvula, was in excellent health after a period of fourteen years. Ringertz reported a seven year cure in a 60 year old man who had had a solitary plasma cell tumor arising from the middle turbinate. Four of the 7 patients with laryngeal tumors were free from recurrence for four years, only 1 of whom had irradiation of the region in addition to surgical treatment. A pharyngeal tumor observed by Ringertz did not respond to high voltage roentgen rays, but after electrocoagulation of the growth the patient remained cured for two years. A nasal tumor reported by Cooper was treated with radium only. While the external swelling of the nose disappeared promptly, a small ulcerated growth was still noticeable in the nasal cavity two years later. The plasma cell tumor at the floor of the mouth which I saw and which measured 2.5 by 1.5 cm. was treated with radium and has remained cured for three and one-half years. Eleven patients of this group were observed after treatment for less than two years—7 had been treated for only six months or less. Two patients died shortly after operation, of bronchopneumonia. No information is given regarding the therapeutic result in 4 cases.

Noncancerous Multiple Tumors.—Nine of the collected cases were instances of multiple plasma cell tumor of the upper air passages without evidence of local invasion or metastases. The patients' ages ranged from 46 to 70 years, and the average age was about the same as in the first group, 58.4 years. Again male patients predominated, 6 to 3. Blacklock and Macartney observed the largest number of tumors in any single patient. Their 64 year old patient, a man, had 5 tumors in the nasopharynx and 2 in the larynx. Wachter found 5 tumors in a 48 year old woman—2 in the nasal cavity, 2 in the nasopharynx and 1 in the larynx. In Heindl's patient a single large mass filled the nasopharynx and extended into both nasal cavities. Three years after it had been removed, 2 new tumors developed in the trachea and 2 in the right bronchus. In addition, a plasma cell tumor was situated in the right upper eyelid. Another case of coexistent conjunctival and tracheal plasmocytoma was described by Kreibig in a 46 year old woman. The 9 patients of this group had in all 33 tumors, 7 of which were in the nasal cavity, 18 in the nasopharynx, 3 each in the larynx and the trachea, and 2 in a bronchus.

The multiple tumors did not differ in their gross appearance from the solitary plasma cell tumors. They were also either lobulated or uniform, polypoid with distinct pedicles or sessile, and their consistency was firm. The color was described as pale gray, sometimes dark brown. They varied in diameter from 0.5 cm. to 4 cm. Few of the tumors were ulcerated or hemorrhagic.

The microscopic picture resembled entirely that of the single tumors. Plasma cells were the principal tumor cells. The majority of observers agreed on the following observations: a slight variation in size and form of the plasma cells and their nuclei; occurrence of two (seldom of more) nuclei; a delicate reticulum demonstrable with silver impregnation, and absence of inflammatory cells. Only in 3 cases did the tumor present a less typical cell structure. Many mitotic figures were noted by Pollock. In regard to the classification of these tumors there was considerable disagreement among the various observers. Six regarded them as noncancerous neoplasms, one (Kreibig) as granulomas, while Heindl was of the opinion that the 4 tumors which developed after excision of the growth in the nasopharynx were implantation metastases. Mattick and Thibaudeau concluded from histologic study that their case was one of low grade cancer.

With respect to the clinical course, the complaints of patients with multiple tumors were the same as those of the former group, only of greater variety. Hoarseness, sensations of pressure, difficulty in breathing and talking were common symptoms, and they were experienced on an average of two years before a physician was consulted. Several patients had these local symptoms for a much longer time—up to twenty years, without impairment of their general health.

Seven of the 9 patients had surgical treatment only; there was recurrence in 3, while 3 others were followed for less than one year. The patient whose case was reported by Wachter had only surgical removal of 5 tumors, and at the time of publication, ten months later, 3 tumors had reappeared. However, eleven years after Wachter's report Oppikofer found that this patient was still living and in good health thirty-one years after the onset of the disease.

Of 2 patients who had surgical and radiation treatment, the first remained well during seven months of observation, but the second, after successful treatment of a nasopharyngeal tumor, had three years later 4 new growths in the trachea and bronchus.

Cancerous Tumors Without Metastases.—Ten of the 64 plasma cell tumors of the air passages showed local destructive properties. The ages of the patients, all men, averaged 62 years and varied between 37 and 77 years. These patients were decidedly older than those with noncancerous tumors.

The principal site of the tumors and probably their source was the mucosa of the nasal cavity. The antrum and the ethmoid sinus were invaded in 8 instances, the sphenoid sinus and the frontal sinus in 3 each, the nasopharynx and the orbital cavity in 2, and the palate and the alveolar process in 1.

Anatomically, the tumors of this group were soft and friable and gray or yellow. The surface was either cauliflower-like or smooth, and sometimes was ulcerated. Hemorrhage was not infrequently seen at the surface or in the deeper portions. Part of the tumor was often necrotic.

Histologically, most tumors of this variety showed more atypical cell structures, a greater variation in size and form of the cells and nuclei, and more mitotic figures than the localized plasma cell tumors did, but these histologic criteria were not always pronounced enough to indicate cancer. For instance, Rosenwasser's case presented clinical evidence of high grade cancer, but in the biopsy specimen the cell structure was quite uniform; many cells had cartwheel arrangement of the chromatin and an eccentric nucleus and gave a typical staining reaction with Pappenheim's method. Likewise, the tumor described by Voegt, which had invaded all the paranasal sinuses and their osseous walls, was composed almost exclusively of typical plasma cells. More atypical pictures were found by Ringertz, Cooper and Baldenweck and Perrot. Besides typical small plasma cells, many large tumor cells were encountered, which resembled lymphoblasts or promyelocytes. According to Ringertz, the reticulum in the cancerous variety of plasmocytoma is much more delicate than that in the noncancerous. Blumenfeld described large areas of necrosis, and Baldenweck and Perrot saw invasion of lymph vessels by tumor cells.

Summarizing the histologic details of these cancerous tumors, we found 4 tumors in which typical plasma cells predominated, 4 others in which only three of the five criteria of the Marschalko type of plasma cell were fulfilled, and 2 with a large number of lymphoblastic cells. However, there was no close relation between the relative maturity of the cells and the destructive capacity of the tumors.

As to clinical course, the patients had symptoms for periods of three months to two years before consulting a physician. While most complaints were similar to those of the preceding groups, pain due to invasion of nerves and hemorrhage with

foul discharge from necrotic areas of the tumor were often additional symptoms. Several of these invasive lesions led to swelling of the nose and cheek, narrowing of the palpebral fissure or lacrimation due to obstruction of the lacrimal duct.

In the attack on these tumors, most radical operations seemed indicated. In 4 cases the whole maxilla was resected, with removal of the ethmoid and sphenoid cells, and in 1 case, in addition, exenteration of the eye. The operative results were disappointing. Three patients died during or soon after operation; 3 tumors recurred after a short time; 1 recurred three times. Radiation was just as unsuccessful. One tumor of Ringertz' series filled both nasal cavities, destroyed the right lateral wall and invaded the skin of the nose and both ethmoid sinuses and the right antrum. Several series of high voltage roentgen ray treatments were given, but the patient died three months later of cachexia. Rosenwasser's patient had plasmocytoma of the nasal cavity with extension to the antrum and to both ethmoid sinuses. Repeated operations and intensive courses of roentgen ray and radium therapy finally arrested the growth. When the patient died, twelve months after the last treatment, the autopsy did not reveal any remnant of the original plasma cell tumor; however, a large carcinoma was found in the stomach, with widespread metastases.

Summarizing the therapeutic results, one finds that 6 of the 7 patients who survived operation died one year or less later. The only patient who lived longer and had apparently no recurrence at the time of publication was the one whose case was reported by Baldenweck and Perrot. The duration of the disease from the onset of the first symptoms in this case was almost twelve years. A small nodule was noticed in the right nasal fossa and increased slowly in size during five years. When it had reached the size of an egg, roentgen examination revealed destruction of the walls of the antrum, of the orbit and of the ethmoid sinus. After radical operation a five year cure resulted. Then the tumor recurred, and after radical operation followed by radium treatment the patient did not show any sign of the disease for one year and seven months.

Cancerous Tumors with Metastases in Lymph Nodes.—Nine plasma cell tumors in the air passages were associated with enlargement of the regional lymph nodes. The average age of the patients, all men, in this group was relatively low, namely, 45.3 years; the youngest patient was 20 years of age; the oldest, 60.

The site of origin of the tumor was the nasal cavity in 4 instances, the pharynx in 2, the tonsils in 3, the larynx in 2 and the tongue in 1. In 7 cases only one of these regions was involved. The majority of the primary tumors had the clinical appearance of destructive lesions and all except 2 were multiple. The lymph nodes were described in 6 cases as well circumscribed tumors which were not adherent to each other. They were firm in consistency and grayish white. The surface was often slightly bosselated, and the cut surface was divided by fibrous septums into fields. Necrotic areas were not infrequent. The size of most lymph nodes varied from that of a pea to that of a walnut. Of unusual size were the tumors of the neck described by Kusunoki and Frank. One of the 20 nodes which had to be removed by operation measured 8 by 4.5 by 3.5 cm.

Histologically, the normal architecture of the lymph nodes was completely obliterated. Only few remnants of lymphoid tissue with small cortical follicles were noticed, and closely packed plasma cells had replaced most of the lymphoid tissue. The tumor cells were never found in the germinal centers of lymph follicles, and there were no recognizable transition forms between lymphocytes and plasma cells. The latter were especially numerous around blood vessels, and several authors, for instance Voegt and Kusunoki and Frank, expressed the belief that the

plasma cells developed from adventitial cells. In 4 tumors the cells were typical plasma cells; in 2 others they did not have all the characteristics of the Marschalko cell, and Ringertz described in his 2 tumors quite atypical cell structures. Only 1 of Ringertz' tumors had a large number of mitotic figures.

In most instances the tumor cells varied in size, and frequently several nuclei were found in one cell. Red-stained hyaline bodies were present occasionally within cells and free in the stroma.

As to the clinical course, the usual duration of symptoms prior to medical treatment was from five weeks to one year. The patient of Bourgeois and Huet had difficulty in breathing for ten years and swelling of the neck for four years before he consulted a physician. Except in the case of Milian, in which excruciating pain was caused by an ulcerated tumor of the tongue, the local complaints were the same as in the former groups. The swelling of the neck was usually found at the time of the first examination or developed several months after removal of the primary tumor. Only in the case of Kusunoki and Frank were the submaxillary nodes noticed one year before nasal obstruction became manifest. In 7 cases only the regional lymph nodes of the neck were involved, while in 2 cases, in addition, the axillary and inguinal nodes were diseased. In 1 case, that of Bourgeois and Huet, only a biopsy was done, and Vogt's patient died without treatment during a sudden attack of suffocation. At autopsy Vogt found large tumors in both tonsils, in the pharynx, in the epiglottis and in the false vocal cords. The cervical, axillary and inguinal glands were enlarged.

Treatment in the other 7 cases consisted of surgical removal in 2 cases, irradiation of the growth in 2 cases and a combination of both in 3 cases. Four of the patients were dead when the cases were reported. Only the following 3 patients survived during the short time of observation: 1. New and Harper's patient, who had a pharyngeal tumor with cervical metastases. This patient was treated with radium and roentgen rays. One year and nine months afterward the primary growth had completely disappeared and the swelling of the neck had diminished in size. 2. Ringertz' second patient, who had a large destructive tumor of the right antrum with rapidly growing metastases in the cervical nodes. The patient was apparently well five months after excision and postoperative irradiation of the site. 3. Borri's patient, who had a plasma cell tumor of the right tonsil and of the nasal mucosa of the left naris, which recurred three times within five years after excision. The last treatment consisted of tonsillectomy and antisyphilitic medication. The patient was reported well one month afterward. The other 4 patients died regardless of the method of treatment. The immediate cause of death was either infection or cachexia. Milian's patient with a lingual tumor succumbed to cellulitis and erysipelas three months after roentgen therapy. Pallestrini's patient died of purulent meningitis thirteen months after combined treatment of a destructive nasal growth. The patient whom Kusunoki and Frank reported as completely recovered one year after surgical treatment died two years after the report was published (Oppikofer). In Ringertz' patient a cervical metastasis developed seven months after combined treatment of a plasma cell tumor originating in the ethmoid labyrinth, and the patient died of cachexia thirteen months after the treatment.

From the clinical and the anatomic standpoint, six observers classified their cases as instances of a malignant neoplasm with true metastases, while three made a diagnosis of granuloma, with tuberculosis (Vogt; Kusunoki and Frank) or syphilis (Borri) as predisposing factors.

Cancerous Tumors with Metastases in Bones.—Nine tumors of the air passages metastasized to bones, and 4 of them, also to lymph nodes. Seven patients were

male and 2 female. The mean age was 48.5 years; the age varied from 39 to 58 years. A fatal outcome was observed in all 9 cases, and since in 5 of them autopsy was performed, a complete anatomic picture of the final stage of this type of tumor is available.

Five of the primary tumors were situated in the antrum, 4 in the nasal cavity, 3 in the ethmoid sinus, 2 in the nasopharynx, 1 in a tonsil and 1 in the larynx. Multiple primary tumors, from 2 to 4 in the same patient, were encountered in 4 cases. Not in every case were the primary growths suspected to be malignant. In the case of Piney and Riach and in the ninth case of Ringertz the nasal tumors were regarded as simple polyps, and in Pollock's case the nasopharyngeal tumor was so small that it had been overlooked before death. Most of the tumors, however, were bulky, sessile, nodular, of soft consistency and partly necrotic. Their destructive capacity was evident on roentgen examination.

The histologic features were similar to those in the preceding group. The uniformity of the cell picture was pointed out by most observers. In 7 cases the nucleus of the tumor cells showed cartwheel arrangement of the chromatin, in 6 it was situated eccentrically, and in 4 instances the cytoplasm stained bright red with Pappenheim's method. Mitotic figures were extremely scanty in some tumors (Jackson, Parker and Bethea; Saarni; Piney and Riach) and abundant in others (Ringertz' cases 1 and 4). Giant cells with two or more nuclei were numerous in the tumors studied by Jackson and co-workers, Ringertz, and Piney and Riach.

The difficulty of making a clinical diagnosis and a prognosis in the presence of such metastasizing tumors is well illustrated in the cases in which the initial tumor either disappeared completely or remained stationary for many years after therapeutic measures, with bone lesions then suddenly becoming manifest and the fatal outcome resulting rapidly. In the first case reported by Jackson and co-workers a plasma cell tumor in a tonsil and the pharynx did not recur after combined treatment for eight and one-half years. Then it reappeared, and at the same time multiple bone tumors were noticed. In Ringertz' fourteenth case a laryngeal tumor that had been surgically removed did not recur. However, after two years tumors composed of plasma cells developed in the skin and in the trachea, and several months later bone lesions could be demonstrated by roentgenogram. The patient observed by Piney and Riach had polyp-like tumors in the nasal cavity and also a mass in the antrum. Combined treatment held the disease in check for twelve years. Then the tumors recurred in the original site and bone lesions became manifest. Death occurred one year later. In Ringertz' ninth case, nasal and paranasal tumors were present for six years before a spontaneous fracture of the humerus disclosed metastasis in a bone. The patient died five months later. In another case reported by this author nasal symptoms caused by an antral tumor were experienced for four years before metastases in lymph nodes and bones became evident.

Three other cases have been difficult to classify, and the question arises whether the tumors were primary growths of the mucosa or metastases of multiple myeloma. Even during autopsy Roessle could not decide whether the bulky destructive mass which he found in the nasopharynx was the primary growth or whether he dealt with multiple myeloma. There were plasma cell tumors in many cervical lymph nodes, in the sternum, in several ribs and in the skull. The history in Roessle's case, with nasal obstruction the chief complaint, would favor the view of a primary tumor of the air passages. Pollock's case is still more problematic. The clinical diagnosis was tumor of the brain, and at autopsy the skull, the sternum and the liver were found to be the seats of plasma cell tumors. A small granular mass composed

exclusively of plasma cells was found at autopsy in the nasopharynx, and Pollock regarded it as the primary tumor. Saarni reported the case of a 39 year old man who had suffered from nasal obstruction for two years. Physical examination showed tumor masses in both nasal cavities, the ethmoid sinus and the right orbit, a supraclavicular lymph node and several ribs at the same time.

While there may be some doubt about the classification of the last 3 cases, the other 6 cases must be regarded as instances of mucosal plasma cell tumor with metastases in bone and not as instances of multiple myeloma; otherwise a latency of medullary tumors up to thirteen years would have to be assumed. The life expectancy of patients with multiple myeloma is given by Geschickter and Copeland as not longer than two years. After bone metastases became manifest in the 6 reported cases, the duration of the disease was from five months to two years, the average being one year and seven months. Of interest are the findings in the urine in 2 cases. Bence Jones bodies were absent as long as only tumors of the air passages were present; however, they appeared in the urine after lesions of bone developed.

CONJUNCTIVA

In 1908 Pascheff described a condition which he called plasmoma of the conjunctiva. He observed 4 patients with swelling of the eyelids due to proliferation of plasma cells. In 2 of these patients trachoma was present, and Pascheff regarded the lesions as inflammatory.

Since then about 50 cases of plasma cell tumor of the conjunctiva have been reported. The following data are based on 29 case reports which were available to me and which present complete clinical and anatomic data. The relatively high incidence of these lesions in females and the occurrence at an early age are in contrast to plasma cell tumors of the air passages. There were 14 female and 13 male patients between 8 and 73 years of age. The mean age was 37.7 years.

Eighteen patients had only one eyelid involved, 10 an upper and 8 a lower lid. In 8 patients two lids were afflicted, either of one or of both eyes. In 3 patients (Baurmann; James; Schwarzkopf) both lids of both eyes were involved. It is interesting to note that 2 of these patients were a 14 year old boy and a 17 year old girl. In 10 cases the mass was firmly attached to the tarsus, in 2 the process had spread to the caruncle, and in the case observed by Verhoeff and Derby it involved the lacrimal sac. In 1 case of Pascheff the tumor covered part of the cornea, and in 3 cases the conjunctiva bulbi was invaded. In 6 cases the tumor originated in the fornix.

Of the 29 cases, 17 were instances of solitary and 12 of multiple tumor. As a rule, the process formed a diffuse swelling or cylindric mass, poorly circumscribed in the conjunctiva. The color was bright red or bluish red; the surface was smooth, seldom papillary or lobulated, and the consistency was firm. A tumor with pedicle was described only by Baurmann, and an elevated strawberry-like mass, by Montpellier. While attachment to the tarsus, to the lacrimal gland or to the lacrimal sac has been observed, the plasma cell tumor of the conjunctiva apparently never has destructive capacities. There is no report of an invasion of the eye itself or of the orbital bones. In Holm's case the orbital plasmocytoma that destroyed the wall of the orbit and was associated with medullary tumors did not arise from the conjunctiva and has to be classified as multiple myeloma.

The microscopic picture was in most instances uniform. The epithelium was well preserved; it was stretched out and thin in some cases and hypertrophic with papillary projections in others. Beneath the epithelium, dense sheets of plasma cells formed the bulk of the tumor. They were often arranged perpendicular to

the surface. No definite capsule separated them from the surrounding tissue, where often thick collars of plasma cells were found around blood vessels. The stroma was in most cases inconspicuous and demonstrable only with silver impregnation. Few observers (Hoffmann) describe a dense stroma with extensive hyaline degeneration.

The tarsus was often invaded and its lamellas split up by strands of plasma cells, while the meibomian glands were seldom involved. Most tumor cells were characterized by an eccentric situation of the nucleus, a cartwheel arrangement of the chromatin, a paranuclear vacuole and a typical Pappenheim stain of the cytoplasm. Large plasma cells with multiple nuclei were not uncommon, and mitotic figures were either absent or scanty. Other cells, as lymphocytes, mast cells and polymorphonuclear leukocytes, did not play any role in the formation of these lesions. Quite often degeneration of the plasma cells was observed. Hyaline globules, the Russell bodies, which stained bright red with Pappenheim's method, were present not only within cells but also free in the stroma.

The hyaline degeneration of the stroma of many conjunctival plasma cell tumors, with the formation of true amyloid in 3 cases, has led to the impression that amyloid and hyaline tumors of the conjunctiva are possibly terminal stages of plasmoma. Pascheff, who first described plasmoma of the conjunctiva, mentioned in 1908 the similarity between plasmoma and amyloid tumor of the eye. Deutschmann went so far as to include plasmoma in the well known entities of hyaline and amyloid degeneration of the conjunctiva.

While it is true that many ophthalmologists (Rachlmann; Kubli; Hubner) have described an intensive plasma cell infiltration in amyloid tumors, others (von Hippel; Kraus; Morax and Landrieu) have not always found it. According to Baurmann, plasmoma may lead to hyaline and amyloid degeneration of the conjunctiva but is not the only disease which precedes it. He found, for instance, that in a large percentage of published cases of hyaline and amyloid degeneration of the conjunctiva the causative agent was a trachomatous infection.

In this connection it is of interest to note that some observers assume an etiologic relation between plasmoma and trachoma. While cultural and inoculation methods with use of fragments from plasma cell tumors have been mostly unsuccessful (Pascheff; James) in support of this theory, it is a fact that in a large number of cases plasmoma is complicated or preceded by trachoma. Most cases of plasmoma have been reported from countries where trachoma is endemic. As Birch-Hirschfeld has pointed out, plasma cells are found in large numbers in trachomatous lesions. Botteri and Spanic inoculated pieces of plasmoma into the conjunctiva of a baboon and produced trachoma. According to Kreibig, in only 5 of the published 35 cases of plasmoma can trachoma be definitely excluded as the etiologic factor. On the other hand, Howard and Saudokoff, of Hongkong, China, were unable to prove any etiologic relation between trachoma and plasmoma, and, they maintained, if the two diseases are associated, it is only a coincidence which is explained by the fact that fully 20 per cent of the population of China are afflicted with trachoma. In 15 of the 30 cases of plasmoma which James collected from the literature the tumor was preceded by trachoma. He feels that present knowledge is insufficient to answer the question of an etiologic relation between these two conditions.

As to clinical course, there is general agreement that conjunctival plasma cell tumors are noncancerous, with very indistinct neoplastic properties. Many ophthalmologists accept the view expressed by Pascheff when he first described this lesion, namely, that the plasmoma is of inflammatory nature. The duration of symptoms—foreign body sensation, reddishness and swelling, but no pain—varied from ten weeks to seven years; most of the patients waited two years before consulting a

physician. The general condition was never affected, and laboratory tests were entirely negative. Excision was the treatment of choice and was in most cases successful. Schwarzkopf and others have pointed out that plasmoma of the conjunctiva may regress or completely disappear in spite of incomplete surgical removal of the tumor. Pascheff reported a two year cure in a 48 year old woman; Schwarzkopf treated a 14 year old boy who for seven years had had plasma cell tumors in all four eyelids. A biopsy after fifteen months did not reveal any tumor tissue. Baurmann reported complete cure lasting several years in 3 patients who had been operated on by von Hippel. Of great interest is the case of Kreibig, which illustrates the different behavior of plasma cell tumors in the conjunctiva as compared with those in the air passages, in spite of identical histologic structure. The patient, a 46 year old woman, had plasmoma of the conjunctiva associated with plasma cell tumor of the trachea. Both lesions were removed by operation. After nine months the growth in the conjunctiva had not recurred, while that in the trachea had recurred and was increasing in size steadily.

Only 2 conjunctival tumors described in the literature recurred after operation. One, which was described by Rund, was situated in the upper eyelid of a 51 year old man. It had been excised fifteen years before Rund saw it and had returned five years later. After a second operation the tumor recurred and was operated on a third time. It measured 2.5 by 1 cm., was hard and consisted exclusively of typical plasma cells.

Tajkef observed recurrent plasmoma of the conjunctiva in a 60 year old woman. Multiple tumors in the left lower eyelid were repeatedly excised and recurred during six years. While the first specimens showed a large number of lymphocytes, the later specimens were more and more composed of plasma cells. There was no evidence of trachoma.

LYMPH NODES

Only 4 cases of plasma cell tumor originating in lymph nodes have been reported. This rarity is surprising if the view of the cytologists is accepted that plasma cells constitute a special type of lymphoid cells. Two of the 4 cases are difficult to classify because they presented widespread bone lesions indistinguishable from multiple myeloma. Only the third case reported by Jackson, Parker and Bethea was regarded as an instance of a localized, noncancerous plasma cell tumor. The patient was a 67 year old, very anemic woman who had a walnut-sized swelling on the left side of her neck. After surgical removal no evidence of recurrence or of metastasis was found during three years of observation. Histologically, the tumor proved to be a plasma cell tumor of a lymph node. If the follow-up period had been longer, the case might have shown a more serious outcome. The other 3 tumors, at least, were much more formidable.

Basset and Scapier observed in a 48 year old man a walnut-sized mass in the sternocleidomastoid region. Two years after onset biopsy established the diagnosis of cancer. In spite of irradiation, the tumor increased in size and became adherent to the skin and fascia. The general condition of the patient also became gradually worse. Roentgen examination showed enlargement of the tracheobronchial lymph nodes, and the differential count revealed 40 per cent mononuclear cells, including few plasma cells. The tumor was partially removed, and postoperative radiation was given. Death occurred six months later, with widespread visceral metastases. Histologically, the lymph nodes were replaced by dense sheets of typical plasma cells, which gave the characteristic metachromatic staining reaction with Pappenheim's method. The nuclei varied in size, and some of the tumor cells had two or more nuclei. Mitotic figures were scanty.

In 1909 Maresch presented before the German Pathological Society a case of "plasma cellular lymphogranuloma." The patient was 48 years of age at the time of death. Eighteen years previously he noticed a small tumor on the left side of his neck. For fifteen years it remained stationary; then it began to increase in size. When the tumor finally reached the size of a fist, it was removed, together with an axillary mass. The tumor in the neck recurred shortly afterward and reached the size of a child's head. Radical dissection of the neck was followed by infection of the operative wound, and the patient died a few days later.

At autopsy no evidence of leukemia was found. There was great swelling of the lymph nodes of the neck and of the axillary regions. The bronchial, mediastinal and retroperitoneal lymph nodes were also enlarged. The inguinal nodes were of normal appearance. The spleen was of double size, but there were no distinct nodules, while the liver was studded with twenty-five well circumscribed nodes varying in diameter from 5 mm. to 1.5 cm. Tumors were also present in the twelfth dorsal vertebra, in the right humerus and in the left femur.

The tumors of the lymph nodes, visceral organs and bones were of identical histologic structure. The main cells were plasma cells, which varied greatly in size and form. They were round, oval or polygonal. The nuclei were either typical, with cartwheel distribution of the chromatin, or had the uniform dark appearance of lymphoblastic plasma cells. Giant cells with one large nucleus or several dark nuclei were frequently encountered. In the small tumor cells many nuclei were pyknotic. Maresch regarded the neoplasm in this case as a type of lymphogranuloma, an opinion which was opposed at the meeting by Sternberg.

While Maresch's case is to be included in this group of extramedullary plasma cell tumors because the tumor of the neck existed for fifteen years without evidence of tumors in bone, the grouping of the second case of Jackson, Parker and Bethea is questionable. Their patient, a 53 year old man, had a hard movable mass in the right side of the neck. It was proved by biopsy to be a plasma cell tumor. At the same time, roentgen examination showed circular areas in the left iliac crest and in the left femur. No Bence Jones bodies were found in the urine. Several months later lesions were disclosed in the skull, ribs, vertebrae and pelvic bones, which in the roentgen picture had the characteristics of multiple myeloma, and a tumor the size of a grapefruit could be felt in the abdomen. The patient died four weeks later; since permission for an autopsy was not given, histologic data on the bone lesions and the abdominal mass are not available.

OTHER ORGANS

Thorax.—Klose in 1911 observed a plasma cell tumor of the pleura in a 61 year old man. The tumor had been excised, together with three ribs; it measured 11 by 7.5 by 5 cm. The surface was reddish brown, and its center was cystic and degenerated. The growth had invaded the intercostal tissue but not the ribs. Microscopic examination revealed almost exclusively plasma cells with all characteristics of the Marschalko type. There was some variation in size and form of the cells, as well as several amitotic divisions, and multinuclear cells were found. The patient died several days after the operation, and a complete autopsy did not reveal any other tumors in the body. Klose believed that this was cancer and that multiple rib fractures which the patient suffered thirty years previously may have stimulated the development of the plasma cell tumor.

A plasma cell tumor in a similar location was described by Bross in 1931. During an autopsy on a 54 year old woman he found a mediastinal tumor which had not given rise to any clinical symptoms. The fist-sized mass was attached

to the posterior surface of the mediastinum and to the first rib on the right side, without invading the bone. The tumor was well encapsulated and consisted of cells which had the morphologic and staining characteristics of plasma cells. Bross did not find any other neoplasms in the body and regarded the mediastinal tumor as noncancerous.

Glandular Organs.—Voegt described, as his fourth case, a case of plasmocytoma of the thyroid. The age and sex of the patient were not given. Urinalysis and roentgen examination excluded the possibility of multiple myeloma. The surgical specimen was a single node, measuring 10.5 by 8 by 5.5 cm. The cut surface was homogeneous and brainlike, and the consistency was soft. Microscopic study did not reveal any remnant of thyroid tissue but showed diffuse proliferation of typical plasma cells. There was a definite paranuclear zone, and the cytoplasm stained red with methyl green–pyronine. Mitotic figures were extremely rare, and the stroma was delicate.

The only other case of plasmocytoma of the thyroid was reported by Shaw and Smith. The patient, a 50 year old woman, had noticed swelling of the neck without toxic symptoms. Roentgen examination showed normal bone structures, and laboratory tests for tuberculosis and syphilis were negative. The thyroid was completely removed and treatment with high voltage roentgen rays given. Examination one year later did not reveal recurrence. The surgical specimen consisted of two lobes, which measured 2 by 4 by 4 cm. and 2 by 4 by 7 cm., respectively. Histologically, only few small acini were preserved between dense sheets of plasma cells. The two authors diagnosed the lesion as a true neoplastic process which had developed in a lymphadenoid goiter.

Lacrimal Gland.—Parker observed in a 66 year old man a plasma cell tumor of the lacrimal gland. The surgical specimen was two and one-half times larger than a normal lacrimal gland; it was lobulated, well encapsulated and grayish brown. Six months after the operation the tumor recurred; it was excised again and the site treated with radium. After ten months the growth recurred a second time and eroded the maxillary bone.

Ovary.—Voegt received for pathologic examination the specimen of an ovarian tumor which had been removed from a 30 year old woman. It was of fist size and cystic on the upper pole. Histologic examination showed masses of plasma cells separated by strands of hyaline connective tissue. The tumor cells stained like typical plasma cells and had all the characteristics except that the paranuclear vacuole was absent in many cells. Mitotic figures were entirely absent. Voegt saw many Russell bodies not only within cells but also free in the stroma.

Intestines.—Brown and Liber presented before the New York Pathological Society a case of multiple plasmocytoma of the small and large intestine. The patient, a 57 year old Negro, had a purulent discharge from the rectum for more than ten years, and there were numerous polypoid firm masses encircling the rectum. He had an old history of syphilis and a positive Frei test. At autopsy numerous masses of yellowish white tissue were attached to the external surface of the ileum, encircling about two thirds of the circumference. The mesenteric and portal lymph nodes were enlarged, and in the hepatic flexure all coats of the colon were invaded by a polypoid mass.

Histologic examination revealed solid sheets of plasma cells with few lymphocytes. Invasion of blood vessels suggested cancer, apparently a variety of lymphosarcoma of multicentric origin.

The following 4 cases of plasma cell tumor in a similar location were cited by Brown and Liber from the literature. Vasiliu and Popa reported a case with

multiple ulcerated nodes in the mucosa of the stomach and intestines. The lymph nodes of the mesentery were invaded. Histologically, all the tumors were made up of plasma cells. Vallone examined a piece of ileum which had been resected from a 24 year old man with chronic intestinal obstruction. The lumen was occluded by a tumor composed of plasma cells. North's case was that of a 47 year old woman who had intestinal obstruction. Thirty centimeters of ileum were resected, and microscopic examination showed marked infiltration of all intestinal layers by plasma cells. Razzaboni reported a case of multiple ulcerated tumors composed of plasma cells in the terminal part of the ileum, the colon and the appendix.

Urogenital Organs.—Knudsen examined a tumor of a kidney which was classified as plasmocytoma and which had produced clinical symptoms of a cancer of the kidney. One year after nephrectomy the patient was in good health.

Ciaccio reported a case of tumor of the spermatic cord of a 38 year old man. The mass had increased to the size of an orange during two and one-half years. After operation no recurrence was observed for eight years. The weight of the surgical specimen was 80 Gm.; it was grayish red and of dense consistency. Microscopic study revealed sheets of plasma cells separated by coarse septums of fibrous tissue. Most of the tumor cells were typical small plasma cells; only few larger cells were present, which resembled lymphoblasts. There were occasional mitotic figures.

Martinotti described a case of plasmocytoma of the vulva. The ulcerated nodules were removed surgically with complete success.

Skin—Aragona saw a tumor composed of plasma cells in the skin of the neck of a 6 year old girl. The patient was followed up for five years after operation, and there was no recurrence in this time.

Hedinger's patient, a 48 year old woman, presented an unusual combination of carcinoma and plasma cell tumor of the skin. She had noticed a small tumor in the scalp since childhood. Ten years before her admission to the hospital, the tumor began to increase in size and became ulcerated. The operation consisted in removal of the primary tumor and two enlarged cervical lymph nodes. The tumor of the skin consisted of two parts. One half was adenocarcinoma originating in adenoma of a sweat gland, while the other half was composed entirely of typical plasma cells. In the cervical nodes only metastatic carcinoma without plasma cells was found. Hedinger interpreted this interesting specimen as representing sarcoma-like proliferation of plasma cells initiated by infection of adenocarcinoma.

THE PLASMA CELL

To understand plasma cell tumors, a knowledge of the normal structure, the function and the origin of the plasma cell is necessary. The literature on the plasma cell is large and controversial. Michels' review, which appeared in the *Archives of Pathology* in 1931, is an excellent source of information.

The plasma cell was first accurately described by Ramón y Cajal in 1890, who encountered it in syphilitic condyloma. He called it the cianophil cell. Unna, in 1891, used the term "plasma cell" for the same cell, which he saw in lupous lesions. This cell was not related to the deep-staining connective tissue cell which Waldeyer in 1875 designated as the plasma cell.

Morphology.—In 1895 Marschalko made extensive observations on normal, pathologic and experimental material, and he gave the following four criteria as essential for the cytologic diagnosis of plasma cells: (1) abundant cytoplasm of

round, oval or polygonal form without specific granuloplasm, (2) eccentric position of nucleus, (3) paranuclear light-stained vacuole, (4) small nucleus with from five to eight distinct deep-staining blocks of chromatin regularly arranged in a circle about the nuclear membrane. For this characteristic feature of the nucleus the term "cartwheel nucleus" was introduced by Pappenheim.

Pappenheim also called attention to a very characteristic staining reaction of the typical plasma cell with methyl green–pyronine. In sections fixed in alcohol (fixation in formaldehyde will often interfere with the stain) the cytoplasm appears bright red while the nucleus takes a bluish green stain. The Pappenheim stain is the most striking characteristic of the plasma cell; however, the pathologist should not diagnose plasma cells from this feature alone. Schmorl pointed out that the cytoplasm of all young cells stains more or less red with pyronine—for instance, Langhans' giant cells, proliferating endothelial cells, syncytial cells of the placenta and the large cells in rheumatic nodules.

Normal Distribution and Origin.—In 1896 Cajal maintained that the plasma cells are normal constituents of the connective tissue and that they originate from tissue lymphocytes; in his opinion, they can transform into fibroblasts. He never saw karyokinesis in plasma cells. Unna expressed the belief that the plasma cells are derived from fixed connective tissue elements and that they divide by amitosis, thereby giving rise to binucleated cells. Marschalko accepted the view of Cajal that plasma cells are normal constituents of the connective tissue; however, he maintained that they are formed from emigrated blood lymphocytes. Large multinucleated plasma cells, giant plasma cells, hyaline globules in the cytoplasm (Russell bodies) are not signs of pathologic growth, but they are found wherever plasma cells occur. Marchand in 1901 derived plasma cells from adventitial cells, the clasmatocytes of Ranvier, and described transition forms between these cells and plasma cells.

Using the modern technic of tissue culture, Maximow in 1922 showed that in explants of lymphoid tissue plasma cells develop from local lymphocytes in the course of two days. He contended that plasma cells are derived from emigrated blood lymphocytes as well as from preexistent tissue lymphocytes. He therefore accepts the opposing views of Cajal and Marschalko in regard to the origin of plasma cells. According to him, wherever an aggregation of lymphocytes occurs, there likewise may be met a varying proportion of plasma cells with transitional stages from lymphocytes. In the interstitial tissue of the glands, the tonsils, the liver and the kidney, in the reticulum of lymph nodes and in that of the bone marrow plasma cells have been shown to be normally present.

Function.—Joannovic in 1899 and Schaffer in 1901 contended that since plasma cells appear wherever there is destruction of nuclei, their formation is due to local absorption of chromatic material and that they help to remove cellular metabolic products. Weidenreich in 1909 expressed the belief that the basophilia of plasma cells is due to a physiologic irritative condition in lymphocytes and that the cells are secretory corpuscles. Huebschmann in 1913 regarded plasma cells as elements capable of elaborating an antitoxic substance. Klein in 1914 and Arneth in 1920 maintained that plasma cells are not degenerating cell forms but functional states of lymphocytes which through local toxic activation are intimately related to immunization processes.

A phagocytic power was ascribed to plasma cells by Nissl in 1904 but was denied by Marchand in 1902 and Greggio in 1909.

Occurrence in Disease.—A marked increase of plasma cells may take place in chronic inflammatory conditions. Tuberculosis, syphilis, encephalitis, trachoma and

subacute gonococcic infection are the most important conditions accompanied with increase in plasma cells. Naegeli in 1919 found 30 per cent plasma cells in the blood of patients with measles.

CLASSIFICATION OF PLASMA CELL TUMORS

If Maximow's view is accepted that plasma cells develop through differentiation of lymphocytes, the tumor composed of such cells has to be classified as a variety of lymphoma. The difficulty, in cases of lymphoma, of distinguishing between an inflammatory and a neoplastic tumor, the uncertainty in correlating histologic structure and clinical course, is familiar to every pathologist. According to Ewing, the complexities of the subject of lymphoid tumors depend on the lack of accurate anatomic classification, and he recommends a rigid application of simple anatomic principles in the classification of these processes.

It would seem that plasma cell tumors with their uniform characteristic cell structure would offer an ideal opportunity to apply the cytologic principles which proved so useful in the study of malignant lymphoma by Gall and Mallory. These authors concluded from a survey of 618 cases that prognostic implications may be guided to a surprising degree by the histologic character of the lesion.

After the literature on extramedullary plasmocytoma has been analyzed, however, it seems that the microscopic appearance does not play such a dominating role in predicting the clinical course of a given lesion. The plasma cell tumor may arise in almost any situation in the body and may behave clinically as a simple benign growth or as a neoplasm of the most malignant type. From a prognostic standpoint, the localization and the gross appearance seem to be more reliable criteria than the histologic structure. For instance, the plasma cell tumors originating in the conjunctiva have such indistinct neoplastic properties that many writers have regarded them as inflammatory, while tumors of identical cytologic character in the air passages may be highly destructive and may produce widespread metastases. In an attempt to correlate histologic structure with clinical behavior I found that, of the 36 noncancerous plasma cell tumors of the air passages, only 26 were composed of tumor cells which corresponded to the Marschalko type while, of the 28 cancerous tumors, 12 had a uniform typical cell structure. In 3 plasma cell tumors of the lymph nodes which were high grade cancers the predominating tumor cell was a typical plasma cell.

The gloomy outlook of some authors, who regard extramedullary plasma cell tumors as fundamentally the same as multiple myeloma, is as unjustified as the opposite view that these tumors are, as a rule, noncancerous processes. If plasma cell tumors are observed for many years, it becomes apparent that as a rule they begin as localized lesions in mucous membranes. This clinically noncancerous phase may persist for a long time, sometimes for ten years or more, but ultimately, without change in histologic character, the process tends to become generalized. Whether or not the dissemination, when it does occur, is the result of metastasis or of multicentric origin of the neoplasm cannot always be stated with certainty.

From the therapeutic standpoint the fact is established that as long as the plasma cell tumor is localized and confined to soft tissue, a clinical cure may be obtained by surgical removal or irradiation of the growth or a combination of both. On the other hand, no therapeutic measure is known to have prevented a fatal outcome if applied after the tumor has either locally invaded bony structures or has spread to lymph nodes or to the skeleton. The uncertainty of prognosis is illustrated by some of these cases, in which the primary tumor had been excised and did not

recur for years, and then unexpectedly secondary foci became manifest in lymph nodes, internal organs or bones and the disease progressed in short time to an unfavorable outcome.

SUMMARY

One hundred and twenty-seven cases of extramedullary plasma cell tumor have been reported since 1905, i. e., approximately 3 cases annually.

In 65 cases the tumor originated in the air passages; in 35 of these it was localized, and in 30 it was definitely invasive. The proportion of men to women was 5 to 1 for the noncancerous lesions and 13 to 1 for the cancerous lesions. The average age of the patients with localized, noncancerous tumors was 57 years, that of the patients with cancerous tumors was 47 years. The duration of the disease was longer than three years in 30 cases, five years in 18 and ten years in 10. At the time of reporting, 23 patients were dead, 4 patients had survived the last treatment for ten years, and 9 patients had lived for three years. After bone lesions became manifest, the duration of survival was one year and seven months. After the tumor had locally invaded bones or had spread to distant organs, no method of treatment prevented an early fatal outcome.

In 50 cases the plasma cell tumor has been reported as originating in the conjunctiva. The sex ratio was 1 to 1, and the average age was relatively low, namely, 37.7 years. In this location the plasma cell tumor has very indistinct neoplastic properties. Only 2 cases are known in which recurrence occurred.

Only in 4 instances did the plasma cell tumor originate in a lymph node. One of the patients survived after operation for a period of three years. While the plasma cell tumor may originate in almost any part of the body, it has actually been found outside of air passages, conjunctiva and lymph nodes in only 13 cases.

The conception of the plasma cell as being a differentiated lymphocyte places the extramedullary plasma cell tumor in the disease entity of lymphoma. A correlation between cytologic appearance and clinical behavior has not been shown. The extramedullary plasma cell tumor, in spite of typical cell structure, may range from an entirely noncancerous growth to a cancer of high grade.

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Forensic Medicine

POISONING BY THE SYNERGISTIC EFFECT OF PHENOBARBITAL AND ETHYL ALCOHOL

AN EXPERIMENTAL STUDY

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Ethyl alcohol (alcohol U. S. P.) and drugs of the barbiturate group rank high among the agents that are commonly responsible for death by poisoning. In view of the frequency with which these substances are ingested it is surprising that there have been so few controlled observations of the extent to which they may exert a synergistic effect. Since each substance when ingested in a sufficiently high dose acts as a respiratory depressant, a combined dosage might reasonably be expected to have an additive effect.

In an active pathologic service concerned with the investigation of medicolegal deaths it is commonly observed that if both alcohol and a barbiturate have been ingested death is likely to occur earlier in the course of the intoxication than it would ordinarily if either substance had been taken alone. Moreover, the observation has been made that when fatal poisoning has resulted from the joint action of ethyl alcohol and a barbiturate, neither agent alone was ingested in sufficient quantity to account for death.

Since the possibility of synergism between alcohol and a barbiturate is a matter of considerable practical as well as theoretic interest, it was decided to undertake an experimental investigation of the problem.

EXPERIMENTAL PROCEDURE

Because of its widespread use, phenobarbital was selected despite the fact that it is more variable in its pharmacologic effects than are most of the other drugs of this group. The procedure adopted was to determine the maximum nonfatal quantity of each substance (ethyl alcohol and phenobarbital) that could be tolerated and then to observe the effect when these amounts or fractions thereof were combined.

The rat is a suitable animal for testing the effects of both ethyl alcohol and phenobarbital. It is widely used in experiments with alcohol and, as shown by Nielsen and others,[1] responds uniformly to barbiturates. Sherman strain rats weighing approximately 100 Gm. were used in all the experiments, and whenever possible the subjects of a single experiment were litter mates.

The maximum sublethal dose of each drug was established. Ethyl alcohol, in a 20 per cent concentration in physiologic solution of sodium chloride, was injected intraperitoneally at the rate of 0.8 mg. per gram of body weight every fifteen minutes until the complete amount had been administered. This method of administration simulated the conditions of a drinking bout. Multiple intraperitoneal injections of equivalent amounts of physiologic solution of sodium chloride produced no immediate or delayed reaction in control animals. In the case of phenobarbital, the sodium salt dissolved in approximately 0.5 cc. of physiologic

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1. Nielsen, C.; Higgins, J. A., and Spruth, H. C.: J. Pharmacol. & Exper. Therap. **26**: 371, 1926.

solution of sodium chloride was injected as a single dose into the subcutaneous tissues of the back. All experiments were carried out with fasting animals.

The gross effect of the drugs when given singly and when given in combination on the animal's activity or on its response to a stimulus was noted. Because frequent and complicated tests would provide an unwanted stimulus, the examinations were conducted as simply as possible. The animals were handled only when injections were made and were kept in separate cages. For simplicity in recording, the following five stages were recognized: (1) normal; (2) muscular incoordination; (3) light unconsciousness; (4) deep coma; (5) death. Coma was differentiated from light unconsciousness by the absence of a reflex when the ear was touched with a fine broom straw.

Alcohol was determined in blood and brain by the unpublished method in use in this laboratory.[2]

Although blood samples obtained by cardiac puncture amounted to only 0.2 cc., the aliquot of the distillate analyzed represented 0.5 cc. The customary human blood aliquot for this procedure is 0.10 cc. The alcohol in the brain filtrate was determined on 0.10 Gm. aliquots.

TABLE 1.—*Determination of the Maximum Sublethal Dose of Ethyl Alcohol in Rats*

Total Dose of Alcohol, Mg. per Gm.*	Animals		
	Used	Survived	Died
4.0	13	13	0
5.0	1	1	0
6.0	1	1	0
7.0	2	2	0
8.0	8	8	0
9.0	4	1	3
10.0	4	0	4

* Alcohol, in 20 per cent concentration in saline solution, was injected intraperitoneally at the rate of 0.8 mg. per gram of body weight every fifteen minutes until the total dose had been given.

TABLE 2.—*Determination of the Maximum Sublethal Dose of Phenobarbital in Rats*

Dose of Phenobarbital, Mg. per Gm.*	Animals		
	Used	Survived	Died
0.10	3	3	0
0.20	8	8	0
0.22	8	3	5
0.24	1	0	1
0.26	1	0	1

* A single subcutaneous injection was made of sodium phenobarbital dissolved in 0.5 cc. of physiologic solution of sodium chloride.

DETERMINATION OF MAXIMUM SUBLETHAL DOSES

Ethyl Alcohol.—Thirty-one rats were used in the experiments. Quantities of alcohol varying from 4 to 10 mg. per gram of body weight were given, with the results shown in table 1. Eight milligrams was the largest amount that could be administered with consistently nonfatal results, since all 8 of the animals to which this amount was given survived. No fatalities were encountered when quantities below this amount were given, in contradistinction to the results when doses greater than 8 mg. were used. Therefore 1 maximum sublethal dose of ethyl alcohol for the animals used in these experiments was 8 mg. per gram of body weight. By our technic ten separate intraperitoneal injections given at fifteen minute intervals completed the administration in two and a quarter hours. With ½ maximum sublethal dose five injections were required and were completed in one hour.

The effect of 1 and of ½ maximum sublethal dose of ethyl alcohol was studied in 6 and in 8 animals, respectively. Typical curves depicting their reactions are shown in chart 1. Muscular incoordination developed within thirty to sixty minutes after the injections were begun. When 1 maximum sublethal dose was administered, the animals ordinarily became

2. The determination is made by measuring the reduction of acid potassium dichromate with a Klett colorimeter, a no. 44 filter being used. The alcoholic extract is a distillate from protein-free Folin-Wu blood filtrate or trichloroacetic acid brain filtrate.

unconscious before the end of the second hour and usually before the complete dose had been given. Deep coma usually followed within a short time thereafter, ordinarily within thirty minutes, and continued for periods varying from one and a half to eight hours. Recovery was gradual with a return to normal in twenty-four hours.

With ½ maximum sublethal dose, there was no progression beyond the stage of muscular incoordination, and a return to normal within eight hours was usual.

In another group of rats, to which 1 or ½ maximum sublethal dose of ethyl alcohol had been administered, the alcohol content of the blood was determined on specimens obtained by cardiac puncture. In no instance were more than two specimens taken from a single animal, nor were these animals included in any other series.

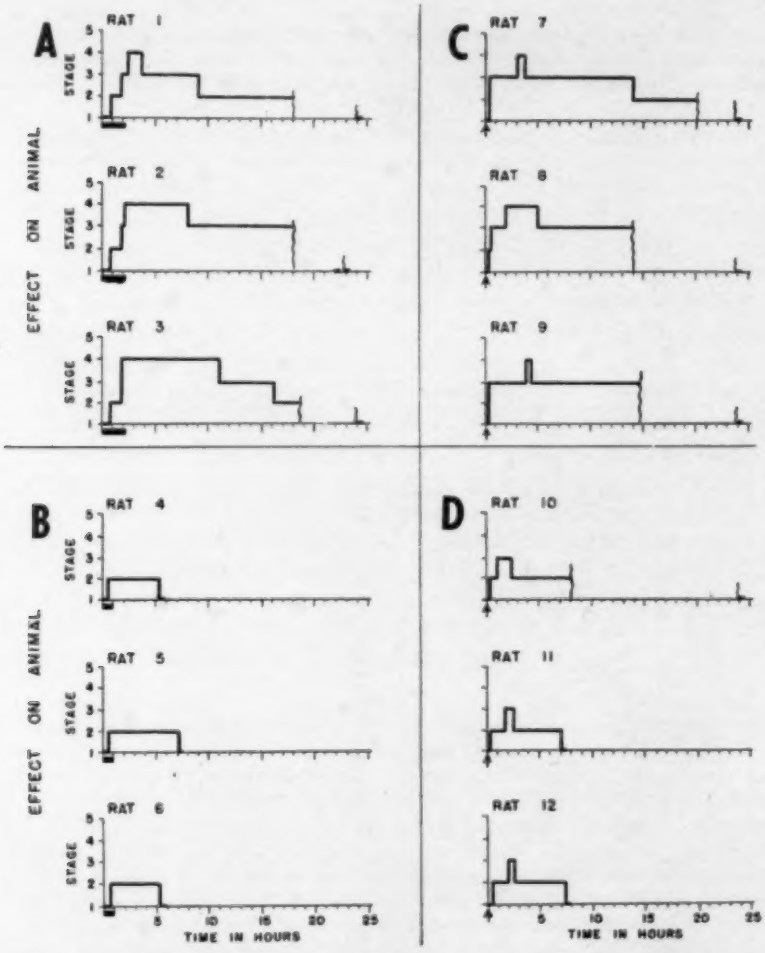

Chart 1.—*A*, effect of the maximum sublethal dose of ethyl alcohol (8 mg. per gram of body weight of rat); *B*, effect of half the maximum sublethal dose (4 mg. per gram). A solid black bar indicates the period of administration of alcohol. *C*, effect of the maximum sublethal dose of phenobarbital (0.2 mg. per gram of body weight of rat); *D*, effect of half the sublethal dose (0.1 mg. per gram). An arrow indicates the time of the injection of phenobarbital.

In stage 1 the rat appears normal. In stage 2 it shows incoordination; in stage 3, light unconsciousness; in stage 4, coma; in stage 5, death.

It will be seen in the case of 1 maximum sublethal dose that the peak of the concentration of alcohol in the blood was reached between three and five hours after the first injection (chart 2). The highest concentration was in the vicinity of 0.75 per cent. With ½ maximum

sublethal dose the maximum concentration of alcohol in the blood occurred between one and a half and two and a half hours after the first injection and approached 0.4 per cent. In both series the alcohol in the blood decreased from the peak to disappear in twenty-four hours.

Phenobarbital.—Twenty-one animals were used in the experiments. A single dose of phenobarbital dissolved in 0.5 cc. of physiologic solution of sodium chloride was injected subcutaneously into the back. As shown in table 2, 0.20 mg. per gram of body weight was the largest amount that could be administered with consistently nonfatal results. All 8 of the animals to which this dose had been given survived, whereas with 0.22 mg. there were five deaths and only three survivals. Therefore, for the animals used in these experiments 1 maximum sublethal dose of phenobarbital was 0.20 mg. per gram of body weight.

The effect of 1 and of ½ maximum sublethal dose of phenobarbital was studied in 6 and in 3 animals, respectively. Typical curves depicting their reactions are shown in chart 1. When the dose administered was 1 maximum sublethal dose, the animals quickly became incoordinated and then, within the next fifteen to thirty minutes, lost consciousness. From one and a half to four hours later all of the animals had become comatose. Coma persisted for periods varying from a half-hour to three hours and subsequently reverted to the stage of light unconsciousness. Twelve hours after the start of the experiment 5 of the 6 animals were still unconscious. Although all appeared drowsy twenty-four hours after receiving the

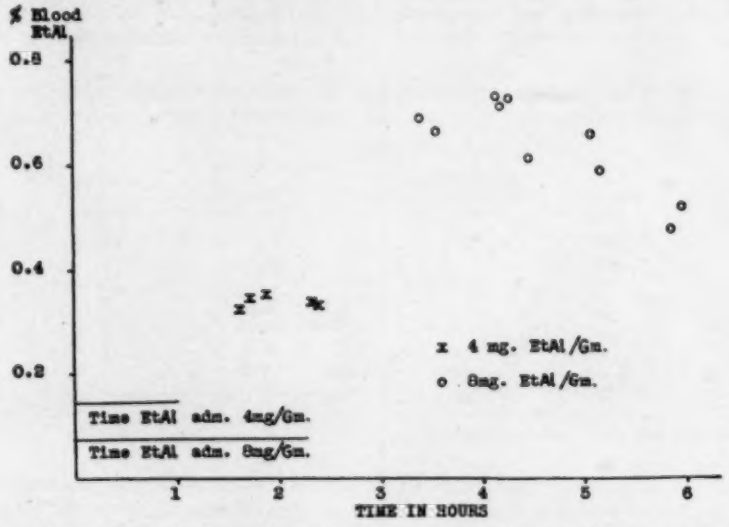

Chart 2.—Values for blood alcohol following the administration of the maximum sublethal dose of ethyl alcohol (8 mg. per gram) to 6 rats (*o*) and half the maximum lethal dose (4 mg. per gram) to 3 rats (*x*). The alcohol, in 20 per cent concentration in saline solution, was injected at the rate of 0.8 mg. per gram of body weight every fifteen minutes.

injection, there was no muscular incoordination, and the results of the examination were recorded as normal.

With ½ maximum sublethal dose, muscular incoordination developed within one-half hour after the injection. This was followed within the next two hours by a period of unconsciousness not exceeding one hour. At no time was there deep coma. Usually the animal returned to normal within a period of eight hours.

EFFECTS OF COMBINED ADMINISTRATION OF ALCOHOL AND PHENOBARBITAL

In order to determine whether or not a synergistic action occurs between ethyl alcohol and phenobarbital, the following combined maximum sublethal doses or fractions thereof were used:

	Alcohol	Phenobarbital
(1)	1	1
(2)	1	½
(3)	½	1
(4)	½	½

The results are given in table 3. Curves depicting the reactions of the individual animals are given in charts 3, 4 and 5.

Combination of 1 Maximum Sublethal Dose of Ethyl Alcohol and 1 Maximum Sublethal Dose of Phenobarbital.—Two different methods of approach were used. In the first phenobarbital was injected at the start of the experiment, simultaneously with the first injection of alcohol. Six rats were used, and all died in from nine-tenths to two and a quarter hours (chart 3). In each instance there was early rapid loss of consciousness, which could be attributed chiefly to the effects of the phenobarbital. Within the next hour coma developed, which terminated fatally soon after. In no instance was the contemplated dosage of alcohol completed. The minimum number of injections of alcohol was three, which was equivalent to a total dose of 2.4 mg. per gram of body weight. The maximum number was nine, which was equivalent to a total dose of 7.2 mg. per gram of body weight.

In all 6 cases the brain, and in 3 the blood, was analyzed post mortem for alcohol. The lowest value for alcohol in the blood was 0.22 per cent and the highest 0.64 per cent. The lowest value for alcohol in the brain was 0.14 per cent and the highest 0.50 per cent. The concentration of alcohol in both brain and blood paralleled the quantity of alcohol administered.

The second method consisted of the administration of phenobarbital following the completion of the injections of alcohol, or two and a quarter hours after the start of the experiment. Six rats were used, and all 6 died (chart 3). The minimum period of survival was three hours and the maximum was five and four-tenths hours. The course to deep coma in two and a quarter hours was that invariably observed when 1 maximum sublethal dose of alcohol was

TABLE 3.—*Effects of Combined Administration of Ethyl Alcohol and Phenobarital in Rats*

Alcohol		Phenobarbital		Animals		
Maximum Sublethal Dose	Mg. per Gm.	Maximum Sublethal Dose	Mg. per Gm.	Used	Survived	Died
1	8.0	1	0.20	6	0	6
1	8.0	1/2	0.10	7	2	5
9/10	7.2*	1	0.20	1	0	1
8/10	6.4*	1	0.20	1	0	1
7/10	5.6*	1	0.20	2	0	2
6/10	4.8*	1	0.20	1	0	1
1/2	4.0	1	0.20	6	1	5
1/2	4.0	1/2	0.10	6	5	1
3/10	2.4*	1	0.20	1	0	1

* Phenobarbital was given before alcohol.

given alone. Deep coma persisted subsequent to the injection of phenobarbital at the two and a quarter hour mark and terminated fatally in periods varying from not less than three quarters of an hour to not more than three and a half hours.

Blood was analyzed post mortem for its alcohol content in 5 of the 6 cases. Concentrations from 0.64 to 0.73 per cent were obtained, indicating that when death occurred the blood alcohol curve was at or close to its peak.

Combination of 1 Maximum Sublethal Dose of Ethyl Alcohol and ½ Maximum Sublethal Dose of Phenobarbital.—The alcohol was given in the usual manner by ten subdivided doses in a total amount equivalent to 8 mg. per gram of body weight, and completion of the dosage required two and a quarter hours. The phenobarbital was given in a single subcutaneous injection immediately after the last injection of alcohol. Six rats were used, and four deaths resulted (chart 4). The course to deep coma in approximately two and a quarter hours was characteristic of that seen with a maximum sublethal dose of alcohol. After the injection of phenobarbital the coma continued for varying periods. Among the animals which died the shortest period of coma was approximately one hour. The postmortem value for the blood alcohol in this case was 0.53 per cent. Probably the blood alcohol at this time was still rising. Two other rats died after approximately ten hours of coma, and the values obtained for the brain alcohol were 0.25 and 0.32 per cent, respectively. In the fourth fatal case the coma persisted for approximately forty hours. The postmortem analysis was negative for alcohol. In the 2 animals which survived, the coma persisted during the period of observation of the first day of the experiment, approximately twelve hours. The next morning, twenty-four hours after the injections, both animals showed incoordination. At the end of forty-eight hours they had recovered completely.

Combination of ½ Maximum Sublethal Dose of Ethyl Alcohol and 1 Maximum Sublethal Dose of Phenobarbital.—In this experiment 4 mg. of alcohol per gram of body weight was

given by five subdivided doses in one hour. As soon as possible after the last injection of alcohol, a single subcutaneous injection of 0.20 mg. of phenobarbital per gram of body weight was given. There were six deaths among the 7 animals which were used (chart 4). At one hour, when the dose of alcohol was completed, muscular incoordination was present. The injection of phenobarbital was followed by rapid loss of consciousness, which progressed to deep coma within forty-five minutes. In the 6 fatal cases death occurred from three-tenths hour to three and a half hours later. Post mortem the concentrations of alcohol in the blood varied from 0.22 to 0.27 per cent.

In the nonfatal case deep coma persisted for nine hours. At the end of twenty-four hours the animal had recovered completely.

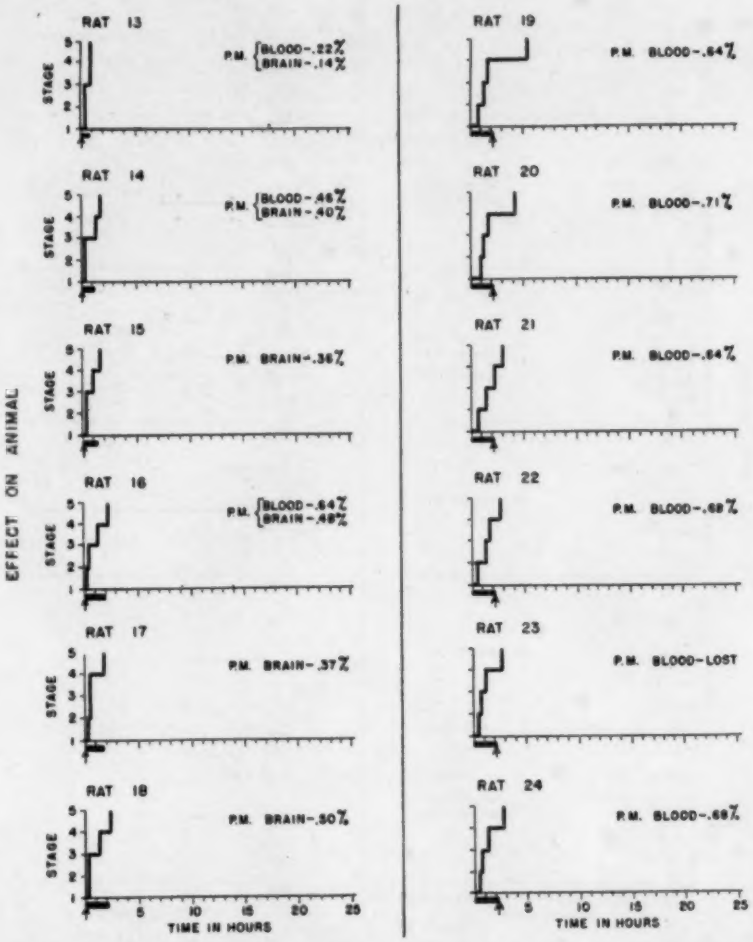

Chart 3.—Effect of the maximum sublethal dose of ethyl alcohol (8 mg. per gram) and the maximum sublethal dose of phenobarbital (0.2 mg. per gram) in combination. A solid black bar indicates the period of the administration of alcohol; an arrow, the time of the injection of phenobarbital. For explanation of the stages, see the legend for chart 1. *P.M.* indicates the values obtained post mortem for the concentration of alcohol in the blood and the brain of the rat.

Combination of ½ Maximum Sublethal Dose of Ethyl Alcohol and ½ Maximum Sublethal Dose of Phenobarbital.—As in the preceding experiment, the administration of ½ maximum sublethal dose of ethyl alcohol was completed in one hour. It was followed immediately by a subcutaneous injection of ½ maximum sublethal dose of phenobarbital. Six animals were used, and one death was recorded (chart 5). At one hour after the dosage of alcohol was completed, the stage of muscular incoordination was present in all instances. Within thirty minutes

after the injection of phenobarbital there was rapid loss of consciousness. In the fatal case coma and death intervened within one and a quarter hours. The postmortem value for blood alcohol was 0.32 per cent. In the 5 nonfatal cases deep coma developed after a period of unconsciousness varying from one-half hour to one and eight-tenths hours and persisted from seven-tenths hour to three and a half hours. All of the animals were normal at the end of twenty-four hours.

COMMENT

The purpose of this investigation was to determine whether or not a fatal synergism develops incident to the combined administration of ethyl alcohol and

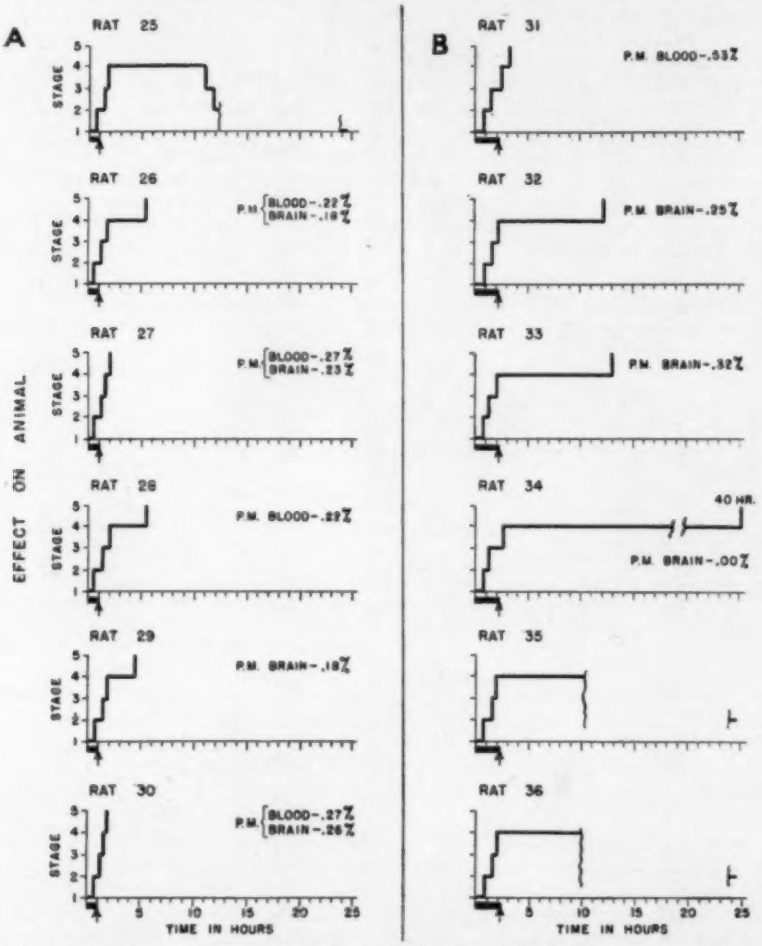

Chart 4.—*A*, combined effect of half the maximum sublethal dose of ethyl alcohol (4 mg. per gram) and the maximum sublethal dose of phenobarbital (0.2 mg. per gram). A solid black bar indicates the period of the administration of alcohol; an arrow, the time of the injection of phenobarbital. *B*, combined effect of the maximum sublethal dose of ethyl alcohol (8 mg. per gram) and half the maximum sublethal dose of phenobarbital (0.1 mg. per gram). For an explanation of the stages, see the legend for chart 1. *P. M.* indicates values obtained post mortem for the concentration of alcohol in the blood and the brain of the rat.

phenobarbital. Some evidence for such a relationship had been demonstrated previously by Dille and Ahlquist [3] in rabbits and by Olszycka [4] in rats. However,

3. Dille, J. M., and Ahlquist, R. P.: J. Pharmacol. & Exper. Therap. **61**:385, 1937.
4. Olszycka, L.: Compt. rend. Soc. de biol. **201**:796, 1935; **202**:1107, 1936.

the dosages used in these investigations were designed to produce sleep and were not extended to levels likely to result in severe intoxication.

Several factors operated to make it impossible to duplicate in the rat the conditions which obtain in man. The first of these was the greater tolerance of the rat for both alcohol and phenobarbital. This difference between the rat and man is only relative since the fatal dose of either drug, though larger, is less than two-fold that for man.

The second factor was the different ways in which the drugs were administered. In human poisoning the drug has usually been taken by mouth; because of vari-

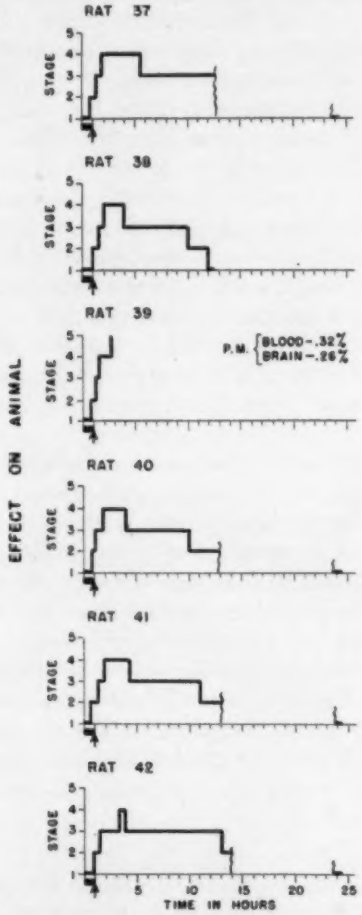

Chart 5.—Effect of half the maximum sublethal dose of ethyl alcohol (4 mg. per gram) and half the maximum sublethal dose of phenobarbital (0.1 mg. per gram) in combination. A solid black bar indicates the period of the administration of alcohol; an arrow, the time of the injection of phenobarbital. For an explanation of the stages, see the legend for chart 1. *P. M.* indicates values obtained post mortem for the concentration of alcohol in the blood and the brain of rat 39.

abilities in absorption of the drug when taken by this route the administration in the experimental animals was by the parenteral route. In the case of ethyl alcohol the administration was accomplished by intraperitoneal injections of subdivided doses, and in the case of phenobarbital, by a single subcutaneous injection. This method of experimental administration has effects essentially similar to those

obtained in man by the oral route. In man alcohol is usually ingested in fractions of the total consumption; on the other hand, in phenobarbital poisoning the total quantity of the drug is usually taken all at once or within a few minutes. The apparent dissimilarity between the continued injection of alcohol into unconscious animals and the cessation of the intake of alcohol with coma in man is not real. The intestinal absorption which continues after the alcoholic human being becomes unconscious is paralleled by our continued intraperitoneal administration of alcohol to the unconscious rat.

The third factor was the difference in at least one particular between the reaction of the rat to the barbiturates, although this is more uniform than that of any other common laboratory animal, and that of man. Death from phenobarbital poisoning in man ordinarily occurs only after prolonged coma, whereas in our experience and that of other investigators death in rats rarely occurs after twenty-four hours. This is an important difference but one that does not invalidate applying these experimental results to explain the mechanism of fatalities induced by the same agents in man.

That there actually was a synergistic action between the two drugs was shown conclusively by the fact that death regularly could be produced by combining non-fatal doses of each drug. Thus, when I maximum sublethal dose of phenobarbital was given simultaneously with the first of the injections of alcohol, the course was so rapid that there was insufficient time to complete a contemplated dose of 1 maximum sublethal dose of alcohol. When 1 maximum sublethal dose of phenobarbital was given at the completion of a series of injections of alcohol equivalent to 1 maximum sublethal dose, death occurred not more than three hours later in all instances.

Further evidence of the synergistic action between phenobarbital and ethyl alcohol was observed when a combination of ½ maximum sublethal dose of each drug was administered to each of the 6 rats. It will be remembered that ½ maximum sublethal dose of either drug given alone produced chiefly muscular incoordination. A progression to the stage of light unconsciousness was not regularly encountered and when present never persisted in excess of one hour. When the dosages were combined, all animals showed severe narcosis, and 1 of the 6 died. The effects were essentially similar to those observed following the administration of a full sublethal dose of either phenobarbital or ethyl alcohol.

The results of combining 1 maximum sublethal dose of phenobarbital with ½ maximum sublethal dose of ethyl alcohol likewise pointed to a powerful synergism. The effect was fatal in 6 of the 7 animals. When the aforementioned doses were reversed, 4 of 6 animals died.

One may conclude, therefore, from the results of this investigation, that a definite synergistic effect occurred in experimental animals to which ethyl alcohol and phenobarbital were administered in combined doses. What appears to have been a similar type of synergistic action has recently been observed in several medicolegal deaths investigated.

CASE 1.—A white man of 35 years was having his hair cut in a barber shop, and although he appeared to be moderately drunk, he was sufficiently cooperative for the barber to proceed without great difficulty. During the twenty to thirty minutes that the deceased was in the barber chair, he was observed to be taking numerous small white tablets, which the barber assumed to be sodium bicarbonate. After the haircut was finished, the man staggered to the door, where he collapsed. He was pronounced dead approximately thirty minutes later.

The autopsy showed no evidence of physical violence. The outstanding abnormalities consisted of severe passive hyperemia, disseminated petechiae and persistently fluid cardiac blood. There was no evidence of a significant quantity of unabsorbed alcohol in the stomach or the intestines.

Postmortem toxicologic studies showed the concentration of the alcohol in the blood to be 0.23 per cent. A large amount of unabsorbed pentobarbital was present in the stomach. A small amount was present in the brain and the blood.

The circumstances in case 1 would be unusual for poisoning by either alcohol or phenobarbital alone. It should be pointed out that the entire sequence of events was acute, death having taken place within one hour after the man had been observed to be moderately intoxicated and one-half hour after he had become comatose.

Could alcohol have been the sole factor in the causation of death? Acute death from alcohol poisoning occurs but is rare. In such instances the postmortem concentration of alcohol in the blood is usually 0.5 per cent or higher and represents the consumption of an inordinately large quantity of liquor within a short time. Such, however, is not the usual manner of death from alcoholic intoxication.[5] Usually death does not occur until several hours and sometimes days have elapsed since the onset of coma. Because of the rapid metabolism of alcohol in the tissues, the level is lower at death than at the antemortem peak. If life persists for twenty-four hours or more after the ingestion of the alcohol, all the alcohol will have disappeared, and chemical analysis will fail to reveal the presence of the fatal poison. In this case because anamnestic data indicated that the death was acute and because the postmortem value for blood alcohol was 0.23 per cent it must be concluded that alcohol was not the sole cause of death.

Could phenobarbital have been the sole factor in the causation of death? Such a rapidly fatal course is not characteristic of barbiturate poisoning, with which death is even more delayed than with poisoning by alcohol. Sometimes the coma is of several days' duration. Moreover, even though the amount of phenobarbital consumed was large, little of it was absorbed. The toxicologic analysis revealed large quantities in the stomach but only traces in the viscera. It may be concluded, therefore, that phenobarbital was not completely responsible for the death.

The most logical explanation for the death of this man is that it resulted from the combined effects of the two poisons. Since it has been established that both substances were present in sublethal quantities and since the manner of death did not resemble that of death from clinical poisoning either by alcohol or by phenobarbital alone, it must be concluded that this death resulted from a synergistic action of the two substances. Presumably this synergistic action depends on the fact that both ethyl alcohol and phenobarbital are depressants of the central nervous system and exert their lethal effect by paralyzing the respiratory center.

Case 2.—A white man of approximately 30 years called a taxicab on a street corner and asked to be taken directly to a hospital. His clothes were wet to a point about half way between the waist and the shoulders, and one of his shoes was filled with coarse gravel. A watch in his pocket was partially filled with water. On being questioned, he stated that he had attempted to drown himself but had given up his attempt before reaching deep water. Prior to the suicidal act he had had several drinks, and just before walking into the water he had ingested a large number of phenobarbital tablets. Because he had changed his mind and now wished to live, he hurried to a hospital for treatment.

At admission he was drowsy. Incompletely dissolved white tablets were obtained by gastric lavage. Efforts to combat deepening unconsciousness were ineffectual, and death occurred approximately six hours later.

The autopsy revealed no evidence of physical violence. As in the foregoing case the chief abnormalities were severe passive hyperemia, disseminated petechiae and persistently fluid postmortem blood, all nonspecific manifestations of a terminal anoxemic state. There were no specific findings to account for death.

The postmortem value for blood alcohol was 0.07 per cent. The following quantities of phenobarbital were present per hundred grams of material: urine, 4 mg.; brain, 5 mg.; liver, 7 mg.; intestinal content, 305 mg.

5. Jetter, W. W.: Clinics **1**:1487, 1943.

While no definite story of the drinking of alcoholic liquor in case 2 was available, there was no evidence to suggest that the man had been drinking for more than a few hours before the unsuccessful attempt at suicide by drowning. In all likelihood the total interval from the beginning of the drinking to death did not exceed nine to ten hours. The concentration of alcohol in the blood at the time of death was 0.07 per cent. Since the man had had nothing alcoholic to drink for the last six hours of life while in the hospital, it is reasonable to assume that for most if not all of this time the alcohol in the blood had been decreasing. Even if it had been decreasing for more than six hours, the peak concentration would still have been far below 0.5 per cent since alcohol metabolizes at an approximate rate of 0.02 to 0.03 per cent per hour.

There are, as in case 1, two objections to pronouncing phenobarbital the sole cause of death. One is that the death was acute, and the other is that although the amount of phenobarbital ingested was great the actual amount absorbed was small. Therefore it must be accepted that death was produced by the combination of the two drugs.

CASE 3.—A white man of 37 years was addicted to both alcohol and barbital. He was last seen alive shortly before midnight, at which time it was said he was drunk. The next morning, approximately eight hours later, he was found dead in bed. There was no evidence of violence.

As in the preceding 2 cases, the anatomic abnormalities were inadequate to explain death and consisted of the usual nonspecific findings incident to terminal anoxemia. Toxicologic analysis showed a postmortem specimen of blood to contain 0.23 per cent alcohol. The following quantities of barbital were present per hundred grams of tissue: liver, 5 mg.; brain, 4 mg.

This case differs from the others in that barbital rather than phenobarbital was recovered by analysis and in that the history was less definite. It is known that the man was found dead about eight hours after he was last seen alive, but it is not known definitely when death occurred or how long death was preceded by coma. Because of the rapidity of the death so far as poisoning by barbital [6] was concerned and because of the low postmortem value for blood alcohol it may be inferred that both drugs contributed to death.

SUMMARY

The synergistic effect between ethyl alcohol and phenobarbital when they are administered in greater than anesthetic doses was demonstrated in a series of rats. When the maximum sublethal dose of each drug was used, death was produced regularly. The maximum sublethal dose of one drug plus half that of the other caused fatalities in a high percentage of animals. Severe narcosis with an occasional death resulted from a combination of half the maximum sublethal dose of one with half that of the other.

Observations resulting from the investigation of three medicolegal deaths occurring in the general community indicate the possibility of similar fatal synergism incident to a combination of the two poisons.

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6. According to the Council on Pharmacy and Chemistry (quoted by Gilman, L., and Goodman, A.: The Pharmacologic Basis of Therapeutics, New York, The Macmillan Company, 1941, p. 139), there is little difference in margin of safety between phenobarbital and barbital.

Notes and News

Personal.—Samuel Soskin has been appointed medical director of Michael Reese Hospital, Chicago. A program of medical teaching will be developed on an intramural basis and gradually extended to the medical profession at large. Dr. Soskin will be the dean of the teaching faculty.

Isaac M. Lewis, professor of bacteriology in the University of Texas, died March 12, 1943, at the age of 65.

Newton J. Evans, professor of pathology of the College of Medical Evangelists, of Loma Linda, Calif., in the vicinity of Los Angeles, has been elected dean of the college.

On his way to take up the deanship of the medical faculty of the University of Teheran, Iran, Charles Oberling, pathologist of the Mary Imogene Bassett Hospital, Cooperstown, N. Y., was forced to turn back on account of illness when he reached Buenos Aires, Argentina. Dr. Oberling has spent two years in reorganizing the medical services of the Iranian government.

Carl Voegtlin, director of the National Cancer Institute since its organization in 1937, will retire on July 31, 1943.

Breeding Center.—The Rockefeller Foundation has made a grant of $35,000 to the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine, in support of a breeding center of rabbits, rats and guinea pigs for scientific research.

Virus Diagnostic Service.—A virus diagnostic unit has been established in the St. Louis Health Division. At present the service will be limited to neurotropic virus diseases.

Awards.—The Sigma Delta Epsilon award of $1,000 has been given to Dorothy M. Ziegler to support her work at the Barnard Free Skin and Cancer Hospital, St. Louis, on epidermal cells, cancerous and noncancerous.

Eli Lilly & Company will continue the Theobald Smith Award ($1,000 and a bronze medal) for at least three years more because the interest in the award has been great and the researches by young scientists, for which it has been given, have been of a high order.

Max M. Strumia, Bryn Mawr, Pa., has been given the Ward Burdick gold medal of the American Society of Clinical Pathologists for his work on the preparation of blood plasma.

Israel Davidsohn, Mount Sinai Hospital, Chicago, received a gold medal for his exhibit on blood groups at the meeting of the American Society of Clinical Pathologists in Chicago, June 3-6, 1943.

Society News.—Walter S. Thomas, Rochester, N. Y., is now president of the American Society of Clinical Pathologists; Frank W. Konzelmann, Wilmington, Del., has been elected president-elect, and A. S. Giordano, South Bend, Ind., secretary-treasurer.

Books Received

DIAGNOSIS OF UTERINE CANCER BY THE VAGINAL SMEAR. George N. Papanicolaou, M.D., Ph.D., department of anatomy, Cornell University Medical College, and Herbert F. Traut, M.D., department of obstetrics and gynecology, Cornell University Medical College and the New York Hospital. Pp. 47, with 11 plates. Price $5. New York: Commonwealth Fund, 1943.

The technic and cellular morphology of human vaginal smears are well presented. The drawings and photomicrographs, all colored, are excellent. As indicated by the title, special stress is placed on the diagnosis of uterine cancer by means of the vaginal smear, but there are chapters also on the epithelial cells found in the vaginal fluid normally and at definite periods of the sex cycle, as well as on the effects of various physiologic and pathologic conditions on these cells. On the jacket the value of the vaginal smear in the diagnosis of cancer is obviously overstated: "The vaginal smear as a means of diagnosing cancer of the uterus was found to be highly reliable, and its routine use disclosed the presence of previously unsuspected cancer." In the text, however, the present diagnostic significance of the smear with respect to cancer is well outlined (page 3): "Two criteria must be fulfilled before the vaginal smear method of diagnosis can be successful or reliable. In the first place, it is not recommended as a means of ultimate diagnosis. It should be used as a preliminary or sorting procedure and should be confirmed as a matter of routine by biopsy and tissue diagnosis. The reasons for this stipulation will become clear to all those who use the method, as they will not infrequently encounter smear preparations which contain so many abnormal cell forms that, while most suspicious of cancer, they are not able to make an absolute diagnosis. In other words, there are many criteria of probable malignancy in the smear preparations in addition to those which may be characterized as absolutely pathognomonic. In the second place, evaluation of individual cells or those arranged in small groups is a much more difficult task, requiring greater knowledge of cytology, than the recognition of cancer in tissue preparations where orientation of the abnormal cells to one another and to the basement membrane is of great assistance in making a diagnosis. In many ways the use of the vaginal smear preparation for the recognition of malignant cells is analogous to the use of blood-smear preparations in the diagnosis of diseases of the blood and blood-forming organs. Patient and repeated search of multiple preparations by well-trained microscopists is essential to success."

MICRURGICAL AND GERM-FREE METHODS. THEIR APPLICATION TO EXPERIMENTAL BIOLOGY AND MEDICINE. A SYMPOSIUM. Edited by James A. Reyniers, director of the laboratories of bacteriology of the University of Notre Dame, Notre Dame, Ind. Volume XII. Pp. 274, with 94 figures and 17 tables. Price $5. Springfield, Ill.: Charles C Thomas, Publisher, 1943.

The section on micrurgical methods contains chapters on micrurgical machines for use in bacteriology (J. A. Reyniers and P. C. Trexler), on micrurgical application of surface chemistry to the study of living cells (M. J. Kopac) and on micrurgy and botany (E. M. Hildebrand). The section on germ-free methods includes an introduction (J. A. Reyniers), the germ-free technic and its application to rearing animals free from contamination (J. A. Reyniers and P. C. Trexler), the use of the mammalian fetus as an experimental animal in bacteriology (O. C. Woolpert and N. Paul Hudson), the germ-free culture of certain invertebrates (R. W. Glaser), germ-free plants and plant parts for physiologic and pathologic studies (P. R. White), the control of cross infection among limited populations (J. A. Reyniers), air conditioning, ultraviolet rays and mechanical barriers in the prevention of cross infection in nurseries (I. Rosenstern and E. Kammerling), air-borne infections and the use of ultraviolet ray barriers (W. F. Wells).

INJURIES OF THE SKULL, BRAIN AND SPINAL CORD: NEURO-PSYCHIATRIC, SURGICAL AND MEDICO-LEGAL ASPECTS. Bernard J. Alpers, Abram Blau, Karl M. Bowman, Samuel Brock, Jefferson Browder, Bronson Crothers, Leo M. Davidoff, C. P. Symonds, Thomas K. Davis, Charles Davison, Cornelius G. Dyke, Charles A. Elsberg, A. R. Elvidge, E. D. Friedman, Francis C. Grant, Clarence C. Hare, George B. Hassin, Paul F. A. Hoefer, Moses Keschner, Max M. Peet, W. Ritchie Russell, Paul Schilder, I. S. Wechsler. Edited by Samuel Brock, New York University. Second edition. Pp. 616, with 78 figures. Price $7. Baltimore: Williams & Wilkins Company, 1943.

The first edition appeared in January 1940 and was reprinted in October 1941. Evidently the book is a useful addition to the literature, medical, surgical and legal, dealing with injuries

of the skull, brain and spinal cord. Important revisions and changes have been made in many of the chapters. Paul F. A. Hoefer is the author of the only new chapter, the electroencephalogram in cases of injury of the head. The contributors, twenty-three in all, represent neurology, psychiatry, surgery, pediatrics and radiology. There can be no question but that the book is paving "the way for a better understanding of the complicated and often controversial situations arising in instances of injury to the central nervous system and for better treatment of the individual patient."

ALLERGY, ANAPHYLAXIS AND IMMUNOTHERAPY, BASIC PRINCIPLES AND PRACTICE: A TREATISE PRESENTING THE FUNDAMENTAL PRINCIPLES AND PRACTICE GOVERNING THE USE OF ANTISERA, VACCINES, TOXOIDS, BLOOD TRANSFUSIONS, BLOOD SUBSTITUTES AND SULFONAMIDES, IN THE PREVENTION AND TREATMENT OF INFECTIOUS DISEASES AND OF THE ALLERGIC PHENOMENA RESULTING FROM THEIR USE. Bret Ratner, M.D., Clinical professor of pediatrics, New York University College of Medicine; visiting pediatrician and director of pediatrics, Sea View Hospital; associate attending pediatrician, Children's Medical Service, Bellevue Hospital; consultant pediatrician, French Hospital. Pp. 834, with 88 figures. Price $8.50. Baltimore: Williams & Wilkins Company, 1943.

The scope of the book is well described on the title page. There are three parts: principles and practice of immunotherapy, including transfusion and the use of sulfonamide compounds; allergy to immunotherapeutic agents; the allergic state—physiologic abnormalities, blood changes and the underlying mechanism. It contains a vast amount of information (not always adequately digested) and will be of interest and help to all who are concerned in one way or another with practical or scientific aspects of sensitization and immunotherapy.

THE AUTOPSY. Journal of Technical Methods and Bulletin of the International Association of Medical Museums, no. XXIII. Special War Number. Robert A. Moore, editor. Pp. 85. Price $2. St. Louis: Washington University School of Medicine, 1943.

A useful outline of the procedures of the autopsy, with descriptions of supplementary methods, prepared by a conference group on pathology, of the National Research Council.

WHOOPING COUGH. Joseph H. Lapin, B. Chem., M.D., Adjunct pediatrician, Bronx Hospital; associate in contagion, Riverside Hospital for Contagious Diseases, New York. Volume XIII. Pp. 237, with 24 plates. Price $4.50. Springfield, Ill.: Charles C Thomas, Publisher, 1943.

The history, epidemiology, etiology, clinical manifestations, diagnosis, prevention and treatment—in other words, all the main phases of whooping cough—receive thorough consideration. The book presents an adequate, up-to-date digest of the present knowledge of whooping cough and is a valuable and timely contribution to the literature on this important disease.

THE ROCKEFELLER FOUNDATION. ANNUAL REPORT, 1942. P. 336. New York: The Rockefeller Foundation, 49 West Forty-Ninth Street, 1943.

NICHOLAS COPERNICUS, 1543-1943. Stephen P. Mizwa, A.M., LL.D. Pp. 88. New York: The Kosciuszko Foundation, 1943.

CORRECTIONS

In the article by Drs. William O. Russell and Ernest Sachs entitled "Pinealoma: A Clinicopathologic Study of Seven Cases with a Review of the Literature," in the June issue (ARCH. PATH. **35**:869, 1943), the legend for figure 2 (which compares the general histologic structure of pinealoma with that of normal pineal tissues) and the legend for figure 3 (which shows the detailed histologic structure of pinealoma) and the references to these illustrations throughout the text are reversed. Mention of these photomicrographs is made on pages 871, 874, 876 882 and 883; in each instance "figure 2" should read "figure 3" and "figure 3" should read "figure 2."

In the article by Drs. Wilhelm C. Hueper and Gustav J. Martin entitled "Tyrosine Poisoning in Rats," in the May issue (ARCH. PATH. **35**:685, 1943), the word "unless" should be substituted for "as long as" in the third line of the last paragraph on page 694.